Universidade Federal do Rio Grande do Sul
Instituto de Ciência e Tecnologia de Alimentos
Programa de Pós-graduação em Ciência e Tecnologia de Alimentos
(PPGCTA)

TESE DE DOUTORADO

Glória Alberto Manhique

AVALIAÇÃO DAS CONDIÇÕES HIGIÊNICO-SANITÁRIAS E CONTAMINAÇÃO MICROBIOLÓGICA DE ALIMENTOS, MANIPULADORES E UTENSÍLIOS UTILIZADOS NA PREPARAÇÃO DE ALIMENTOS EM MERCADOS E NAS RUAS DE MAPUTO, MOÇAMBIQUE.

Porto Alegre
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Orientador: Prof. Dr. Eduardo Cesar Tondo

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Tese de Doutorado

Avaliação das condições higiênico-sanitárias e contaminação microbiológica de alimentos, manipuladores e utensílios utilizados na preparação de alimentos em mercados e nas ruas de Maputo, Moçambique.

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RESUMO

Nos últimos anos, o número de refeições consumidas fora de casa tem aumentado, bem como o número de surtos alimentares envolvendo essas preparações e os locais que às produziram. Em Maputo, Moçambique, muitas refeições são realizadas em estabelecimentos como mercados públicos e ruas, sendo que muitos deles não apresentam condições higiênico-sanitárias adequadas. Dessa forma, o primeiro objetivo deste trabalho foi avaliar as condições higiênico-sanitárias de preparo de alimentos em estabelecimentos de sete mercados públicos da cidade de Maputo. Para tanto, utilizou-se um *checklist* constituído por 36 questões relativas às práticas de higiene e manipulação de alimentos, o qual foi submetido a 190 manipuladores de 190 estabelecimentos preparadores de alimentos de sete mercados de Maputo. O *checklist* foi elaborado com base no Diploma Ministerial n. 51 de 3 de outubro de 1984, que consiste na legislação utilizada para avaliar às condições higiênico-sanitárias de estabelecimentos de alimentação em Moçambique. Para classificar os estabelecimentos quanto às condições higiênico-sanitárias, foi criado o *Food Safety Index* (FSI). Esse índice permitiu classificar cada estabelecimento em três níveis de adequação das condições higiênico-sanitárias (inadequadas, satisfatórias e adequadas), e o índice foi calculado utilizando a média harmônica ponderada onde foram considerados pesos diferentes para cada grupo de questões relacionadas às condições higiênico-sanitárias dos estabelecimentos. O resultado dos *checklists* demonstrou que, nos mercados, a maioria (85,76%) dos estabelecimentos apresentou um FSI entre 0,15 e 0,18, tendo sido classificados como em condições inadequadas ou ruins. O segundo objetivo do estudo foi avaliar o nível de conhecimentos e atitudes sobre segurança de alimentos de manipuladores e investigar *Enterobacteriaceae* nas mãos dos mesmos e nas saladas de alface prontas para consumo, vendidas nas ruas da cidade de Maputo. Ao todo, 110 manipuladores de alimentos foram entrevistados e 55 amostras de saladas e 55 amostras de mãos foram analisadas, utilizando o método ISO 21528-2 para *Enterobacteriaceae.* A maioria das pessoas entrevistadas foi do sexo feminino, com menos de 35 anos e nunca tinha recebido treinamento em segurança de alimentos. Mais da metade (58%) dos entrevistados possuía conhecimento insuficiente sobre segurança de alimentos, principalmente em relação à contaminação cruzada, controle de temperatura e patógenos alimentares. A contagem média de *Enterobacteriaceae* nas saladas prontas para consumo e nas mãos dos manipuladores foi de 4,23 ± 0,78 log cfu/g e 3,14 ± 0,7423 log cfu/g, respectivamente. *Escherichia coli* genérica foi detectada em apenas uma amostra de mãos e não foi observada correlação significativa positiva ($P < 0,05$) entre os conhecimentos e atitudes e os microrganismos encontrados. O terceiro objetivo desse estudo foi avaliar a prevalência de *Enterobacteriaceae* em amostras de água, saladas de alface prontas para o consumo, mãos dos manipuladores e utensílios dos mercados da cidade de Maputo e, para tanto, 182 amostras foram analisadas. A identificação dos isolados foi realizada com o auxílio de espectrometria de massas (MALDI-TOF) e aquelas que foram positivas para *Escherichia coli*, foram submetidas à análise de reação em cadeia da polimerase (PCR) para a presença de genes de virulência (*stx1F, stx1R, stx2F, stx2R*). Dentre os 219 isolados obtidos, *Enterobacter* spp. foi a espécie predominante (45,2%). A contagem de *Enterobacteriaceae* variou de 0,52 a 6,98 log UFC /g e a presença de *E. coli* foi observada em 13 amostras,

as quais foram negativas quanto à presença de genes de virulência. A partir dos resultados obtidos, constatou-se a necessidade de treinamentos e inspeções nos estabelecimentos que preparam alimentos nos mercados e ruas de Maputo, dado que deficiências nos conhecimentos e nas condições higiênico-sanitárias foram constatadas com frequência. A contaminação por *Enterobacteriaceae* indicou possíveis falhas nos procedimentos de higienização ou contaminação cruzada, contudo a baixa prevalência de *E. coli* e a ausência de genes de virulência nesses microrganismos indicaram que outros fatores colaboram com a baixa contaminação fecal ou com a sobrevivência desses microrganismos. Outros estudos são necessários para compreender tais resultados.

Palavras chaves: vegetais contaminados, manipuladores de alimentos, segurança de alimentos, mercados públicos, vendedores de rua

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

ANVISA Agência Nacional de Vigilância Sanitária

BP Boas Práticas

DTA Doenças Transmitidas por Alimentos

RMO Requisitos Mínimos Obrigatórios

OMS Organização Mundial da Saúde

1. INTRODUÇÃO

O aumento no número de refeições consumidas fora de casa tem sido observado no mundo todo e isto ocorre devido às mudanças no estilo de vida resultantes da globalização (WHO, 2015). De acordo com Saad (2017), famílias americanas gastam mais de 50% do seu orçamento alimentar em refeições fora de casa, enquanto, no Brasil, o setor de restaurantes e bares tem registrado um crescimento anual em torno de 10%. O mesmo pode ser observado no Canadá onde, no ano de 2016, mais da metade (54%) da população respondeu ter o hábito de se alimentar fora de casa, pelo menos, uma vez por semana (CANADA, 2016).

Paralelamente a isso, dados crescentes de Doenças Transmitidas por Alimentos (DTA) sugerem que os alimentos preparados fora de casa são uma fonte importante de contaminação (JONES; ANGULO, 2006). Nos EUA, por exemplo, os restaurantes são considerados os locais de maior ocorrência de surtos, tendo sido responsáveis pela ocorrência de 61% dos surtos registrados entre os anos 2009 à 2015 (DEWEY-MATTIA et al., 2018; MUN, 2020). No Brasil, dados da Secretaria de Vigilância em Saúde, do Ministério da Saúde, demonstram que os restaurantes são o segundo lugar de ocorrência de surtos de DTA registradas, ficando atrás apenas das residências (BRASIL, 2017).

Alimentos de rua são definidos como alimentos e bebidas preparados e/ou vendidos nas ruas e em outros locais públicos para consumo imediato ou posterior, sem que haja etapas adicionais de preparo ou processamento. Esta definição inclui frutas e vegetais frescos vendidos fora das áreas de mercado autorizadas para consumo imediato (WHO, 1996).

Quando as condições são inapropriadas para preparação de alimentos, eles podem veicular patógenos causadores de doenças. Os alimentos vendidos em mercados públicos e ruas também podem não ser seguros, dado que nesses locais muitas vezes as condições são inadequadas. Outro fator importante é a falta de conhecimento sobre assuntos de segurança de alimentos dos manipuladores. Nesses locais de venda, os alimentos, podem não ser adequadamente tratados termicamente e as temperaturas de conservação e distribuição podem ser inadequadas, o que aumenta o risco de

ocorrêcia de DTA (CARDAMONE et al., 2015; MIR et al., 2018; TAMBEKAR; MUNDHADA, 2006; WHO, 2019).

Nos últimos anos, tanto nos restaurantes como na alimentação vendida nas ruas, o consumo de saladas de vegetais aumentou drasticamente, devido aos benefícios à saúde atribuídos ao seu consumo (CALLEJÓN et al., 2015; GUSTAT et al., 2015; TOPE; HITTER; PATEL, 2016) e na praticidade em sua preparação. Paralelamente a isso, o número de surtos relacionados ao consumo de saladas de vegetais frescos também aumentou (BERG et al., 2014; SHAPIRO et al., 2019) e uma das razões para isso é a falta de tratamento térmico durante a sua preparação (CARDAMONE et al., 2015; TAMBEKAR; MUNDHADA, 2006) ou a falta de condições higiênico-sanitárias, desde a produção até o consumo. Corroborando esses fatos, no período de 2010 a 2017, houve registro de 85 surtos relacionados a vegetais frescos com etiologia confirmada nos Estados Unidos (CARSTENS; SALAZAR; DARKOH, 2019; SELF et al., 2019).

Atualmente, cada vez mais informações apontam que vegetais frescos folhosos são importantes reservatórios de *Enterobacteriaceae* patogênicas e multirresistentes, sendo considerado um importante problema de saúde pública (ESTEBAN-CUESTA et al., 2019; RICHTER et al., 2019; USUI et al., 2019). A família *Enterobacteriaceae* é um grupo de bactérias gram-negativas, não esporuladas. Nesta família, estão inclusas muitas bactérias residentes no trato intestinal humano ou animal, além de plantas e ambiente (CFS, 2014). As *Enterobacteriaceae* podem apresentar vantagens sobre os coliformes como indicadores de condições higienico-sanitárias e de boas práticas, uma vez que possuem coletivamente maior resistência ao ambiente (BERNASCONI; DAVERIO; GHIANI, 2003; CFS, 2014), além de representarem mais de 50 espécies bacterianas, ao contrário dos coliformes que são representados por, basicamente, cinco espécies. Nesta família estão incluídos importantes patógenos alimentares como *Cronobacter spp*, *Escherichia coli*, *Salmonella enterica*, *Shigella* e *Yersinia* (BAYLIS et al., 2011; PATEL et al., 2014; SMITH; FRATAMICO, 2015).

Em Moçambique, existem diferentes serviços de alimentação, sendo que os muitos estabelecimentos estão localizados nos mercados públicos, vendedores de ruas e pequenos restaurantes. Estudos sobre as condições

higiênico-sanitárias, bem como a qualidade microbiológica dos alimentos comercializados nesses locais são escassos, porém são muito importantes, pois permitem acessar às condições encontradas em Moçambique e compará-las com às condições encontradas em outros países, assim como confrontá-las com os padrões microbiológicos estabelecidos pela legislação local ou internacional. As análises microbiológicas realizadas no presente estudo, especialmente das saladas de vegetais prontas para o consumo, foram realizadas, tendo em consideração o fato de que as mesmas não sofreram tratamento térmico e podem não terem sido higienizadas adequadamente, nos estabelecimentos localizados nos mercados e nas ruas de Maputo. Esses fatos aumentam a possibilidade de existência de membros da família *Enterobacteriaceae*, os quais podem servir como indicadores das condições higiênico-sanitárias desses locais.

2. OBJETIVOS

2.1 Objetivo Geral

Avaliar as condições higiênico-sanitárias e a contaminação microbiológica de alimentos, manipuladores e utensílios utilizados na preparação de alimentos em mercados e nas ruas de Maputo, Moçambique.

2.2 Objetivos específicos

a) Avaliar as boas práticas de preparo e venda de alimentos nos mercados públicos na cidade de Maputo;

b) Avaliar os conhecimentos sobre segurança de alimentos dos vendedores de comida de rua na cidade de Maputo;

c) Investigar a prevalência de *Enterobacteriaceae* em saladas de alfaces prontas para o consumo, mãos dos manipuladores superfícies e água utilizadas para a preparação de alimentos nos mercados da cidade de Maputo;

d) Investigar a presença de genes de virulência em *E. coli* isoladas de amostras de alfaces e saladas prontas para o consumo vendidas na cidade de Maputo.

3. REVISÃO BIBLIOGRÁFICA

3.1 Doenças Transmitidas por Alimentos

Doenças Transmitidas por Alimentos, mais comumente conhecidas como DTA, são todas aquelas doenças causadas pela ingestão de alimentos e/ou bebidas contaminados por microrganismos e/ou suas toxinas ou produtos químicos, constituindo um grande problema de saúde pública, em nível mundial (NUNES et al., 2017; OLIVEIRA et al., 2010; WHO, 2017). De acordo com o Centro de Controle e Prevenção de Doenças (CDC), existem mais de 250 DTA e a sua maioria são infecções causadas por bactérias e suas toxinas, vírus e parasitas. Os sintomas mais comuns das DTA são náuseas, vômitos, cólicas abdominais e diarreia, podendo ser graves e, em alguns casos, fatais (CDC, 2017a; WHO, 2018).

Surto é um episódio em que dois ou mais indivíduos apresentam os mesmos sintomas, após ingestão de alimentos de mesma origem (MINISTÉRIO DA SAÚDE, 2010). Para o caso de patógenos altamente virulentos, como *Clostridium botulinum* e *Escherichia coli* O157:H7, assume-se que apenas um caso pode ser considerado um surto (FDA, 2020).

A Organização Mundial da Saúde (OMS) estima que, a cada ano, o consumo de alimentos contaminados cause 600 milhões de casos de DTA e 420.000 mortes, em todo o mundo, sendo as crianças menores de cinco anos as mais afetadas, perfazendo cerca de 125.000 mortes (WHO, 2020). Por outro lado, o Centro de Controle e Prevenção de Doenças (CDC), estima que 48 milhões de casos de DTA ocorram a cada ano, nos Estados Unidos, resultando em aproximadamente 128.000 hospitalizações e 3.000 mortes (DEWEY-MATTIA et al., 2018). No Brasil, a Secretaria de Vigilância em Saúde, registrou, em 2018, 598 surtos de DTA, causando doenças em 9320 pessoas e 12 óbitos (BRAZIL, 2018). Entretanto, o número preciso de DTA que ocorrem no mundo, a cada ano, pode ser apenas estimado, devido ao grave problema de subnotificação, pois muitas vezes os sintomas dessas doenças são brandos, fazendo com que a maioria das vítimas não busque auxílio médico e, consequentemente, as autoridades não sejam informadas (FORSYTHE, 2013; SCALLAN et al., 2011; STANWELL-SMIHT, 2017).

Dados sobre o número de casos de surtos e/ou DTA que ocorrem em Moçambique são escassos, assim como acontece em outros países em desenvolvimento, entretanto sabe-se que o risco de ocorrência de surtos e/ou DTA é maior nesses países (WHO, 2017). Em Moçambique, mais de 500 mil pessoas tiveram doenças causadas pelo consumo de alimentos inseguros em 2018 (INSTITUTO NACIONAL DE ESTATISTICA, 2018). Segundo a ONU, estes casos correspondem apenas a 5,5% dos casos de diarreia detectados e notificados (POTA, 2019). Dados do Ministério da Saúde de Moçambique demonstram que, desde Janeiro até a primeira semana do maio de 2020, o país notificou 492.152 casos e 198 mortes, devido a ocorrência de doenças diarreicas (MOÇAMBIQUE, 2020).

Dentre os patógenos alimentares que afetam milhões de pessoas mundialmente, pode-se destacar *Salmonella*, *Campylobacter* e *Escherichia coli* entero-hemorrágicas (WHO, 2020). O CDC estima que entre as 9,4 milhões de doenças causadas por 31 patógenos conhecidos, 90% delas são causadas por sete patógenos: *Salmonella*, Norovirus, *Campylobacter*, Toxoplasma, *E. coli* O157:H7, *Listeria monocytogenes* e *Clostridium perfringens* (CDC, 2017b). Uma revisão de registros de DTA na Índia, no período de 1980 a 2016, mostrou que Norovirus, *Vibrio* spp., *Salmonella* spp., *E. coli*, *Staphylococcus aureus e Yersinia enterocolitica* foram os microrganismos mais envolvidos nos surtos (ALIMI, 2016). Associado a isso, McDaniel e Jadeja (2019) realizaram uma revisão de novos surtos, preocupações e intervenções atuais com a segurança de alimentos nos Estados Unidos, e concluíram que o número de DTA está aumentando, sendo que *E. coli*, *Salmonella* spp. e *L. monocytogenes* estão entre os patógenos mais comuns como causadores de doenças significativas nos humanos.

No caso concreto de Moçambique não se sabe ao certo os principais patógenos envolvidos nos casos de DTA, entretanto o *Vibrio cholerae* continua sendo uma preocupação. Em 2015, foram registrados 3.500 casos de cólera, em três pronvicias de Moçambique, tendo resultado em um total de 37 mortes (MÉDICOS SEM FRONTEIRAS, 2016). Recentemente no ano de 2019, foram registrados mais 700 casos de cólera e 109 óbitos em províncias do país. Preparo de alimentos, muitas vezes, com água contaminada, infraestruturas precárias nos locais de preparação, assim como condições inadequadas de

conservação dos alimentos, são considerados fatores importantes que contribuem para o aumento de casos de DTA, nos países de baixa renda, como é o caso de Moçambique (ANDRADE, 2008; TRAFIALEK; DROSINOS; KOLANOWSKI, 2017; WHO, 2011).

3.2 Condições Higiênico-sanitárias de Preparo e Venda de Alimentos nos Mercados e Ruas

O consumo de alimentos de rua está se tornando um fenômeno crescente, porque constitui uma alternativa econômica e prática para a população. Os alimentos de rua geralmente refletem às culturas locais tradicionais e existem em uma variedade infinita. Há muita diversidade nas matérias-primas, bem como nas suas preparações. Segundo a OMS, comida de rua é definida como os alimentos e bebidas preparados e/ou vendidos pelos vendedores nas ruas e em outros locais públicos para consumo imediato ou posterior, sem que haja posterior processamento ou preparação. Esta definição inclui frutas e vegetais frescos vendidos fora das áreas de mercado autorizadas para consumo imediato (WHO, 1996). Os mercados públicos e ruas são locais onde se expõem e se comercializa grande variedade de alimentos (DE SOUZA et al., 2014; FRANCO; UENO, 2010). Para o caso concreto de vendedores ambulantes, eles se localizam em áreas com grandes fluxos de pessoas como: centros comerciais, feiras, praças, pontos de ônibus, táxi, escolas, entre outros. Dentre os alimentos comercializados nestes locais podem estar carnes, arroz salgados, cachorro-quente, hambúrgueres, pastéis, batata fritas, saladas de vegetais, frutas além de outros alimentos perecíveis e envolvidos em surtos de DTA.

As preocupações sobre a segurança destes alimentos são cada vez maiores, devido à falta de infraestrutura e serviços básicos, como abastecimento de água potável, dificuldade em controlar o grande número de operações de venda, recursos insuficientes para inspeção e análise laboratorial; falta de conhecimento sobre segurança de alimentos por parte dos manipuladores (WHO, 1996). Estes fatores podem contribuir para o surgimento de DTA, as quais afetam a saúde e a integridade dos consumidores.

Muitos estudos indicam que as condições higiênico-sanitárias dos locais de venda de alimentos, nos mercados e ruas, não são adequadas, podendo constituir um problema à saúde do consumidor. Por exemplo, Magalhães et al. (2017) analisaram às condições higiênico-sanitárias de locais de comércio ambulante de alimentos, no município de Umuarama, Brasil, e concluíram que em relação às edificações e instalações, manipuladores e produção de alimentos, 63% apresentam não-conformidades com a legislação. Kipper et al. (2019) verificaram as condições higiênico-sanitárias de *food trucks,* no estado de Santa Catarina, Brasil, por meio da utilização de um *checklist* baseado nas RDC 275/2002 e 216/2004 e observaram que 100% dos *food trucks* tinham ausência de proteção contra insetos nas janelas e aberturas, falta de separação de área de preparo dos alimentos e pré-preparo e ausência de sanitários exclusivos aos funcionários. Em relação aos manipuladores, 84% não utilizavam uniformes adequados e 61% não estavam com o cabelo protegido e com barba aparada. Os autores concluíram que às condições higiênico-sanitárias dos *food trucks* eram insatisfatórias. Sobral et al. (2013) avaliaram às condições higiênico-sanitárias do mercado público da cidade de Russas, Ceará, a fim de observar os elementos de qualidade e segurança, e verificaram que as edificações, os equipamentos e materiais, os manipuladores, processo e transporte dos alimentos encontravam-se de forma precária, com um percentual de inadequação média de 82%, tendo sido classificados como em más condições. Nesta perspectiva, devido à possibilidade de contaminação microbiológica que possivelmente pode ocorrer em condições de rua, é necessário direcionar esforços para educar o pessoal envolvido; melhorar às condições ambientais em que a preparação e o comércio de alimentos são praticados e fornecer os serviços essenciais para ajudar os vendedores ambulantes de alimentos a garantir a segurança de seus produtos.

3.3 Tipos de serviços de alimentação de Moçambique e suas características

Ao longo dos tempos, o comércio de alimentos prontos para o consumo tem sido uma estratégia de sobrevivência, na medida em que minimiza os principais problemas estruturais dos centros urbanos, contribuindo para

aumentar a oferta de trabalho, principalmente, para mulheres com baixa renda, em países em desenvolvimento, como é o caso de Moçambique (WHO, 2006).

Apesar das vantagens, em muitos países africanos, o setor de venda de alimentos enfrenta vários problemas. Estudos têm demonstrado que as condições higiênico-sanitárias e físico-estruturais do comércio de alimentos em países em desenvolvimento são precárias, comprometendo à saúde dos consumidores (DE SOUZA et al., 2014; FRANCO; UENO, 2010; MAGALHÃES et al., 2017). Serviço de alimentação é o estabelecimento onde o alimento é manipulado, preparado, armazenado e ou exposto à venda, podendo ou não ser consumido no local (BRASIL, 2004). Em Moçambique, fazem parte de serviços de alimentação as cantinas, padarias, pastelarias, restaurantes, estabelecimentos em mercados e ambulantes. Contudo, a venda de refeições prontas para o consumo na cidade de Maputo é realizada, na sua maioria, em três locais principais: barracas (como são chamados os serviços de alimentação localizados nos mercados), ambulantes e restaurantes. Estes estabelecimentos geralmente estão situados em locais onde há concentração elevada de postos de trabalho, proximidades de paradas de transportes públicos, escolas, hospitais, entre outros.

Os mercados em Moçambique são definidos como locais fechados ou não, nos quais múltiplos vendedores promovem a venda ou facilitam a aquisição de produtos aos consumidores, bem como a prestação de determinados serviços permitidos por lei. Eles estão sobre administração do Conselho Municipal ou de outro órgão, em regime de concessão ou parcerias Público-Privadas (CONSELHO MUNICIPAL DE MAPUTO, 2008).

Nos mercados e vias públicas de Moçambique, a venda de alimentos insere-se no mercado de trabalho informal. Nestes locais, muitas vezes, os alimentos são expostos em contato direto com o chão, o qual pode ser de areia ou de cimento, ou colocados por cima de caixas de madeiras ou plásticos, sendo, frequentemente, vendidos próximos dos depósitos de resíduos sólidos. Além disso, nota–se também a existência de águas estagnadas e de fossas (MMO, 2015).

No ano de 2017, a INAE (Inspeção Nacional das Atividades Econômicas), órgão responsável por realizar a inspeção dos estabelecimentos alimentares, em Moçambique, fechou 30 viaturas de venda de alimentos em

ruas e passeios na cidade de Maputo. Um total de 20 restaurantes também foi encerrado, no mesmo período. Nos restaurantes foram detectadas as seguintes irregularidades: fossas entupidas, cozinha e sanitários sujos, concentração de águas negras, na forma de charcos, que exalavam cheiro nauseabundo para o interior da cozinha, má conservação do refeitório dos trabalhadores, paredes com infiltração de água e presença de gatos, nas instalações (FOLHA DE MAPUTO, 2017).

Mediante a negligência de alguns órgãos públicos e tendo em vista a necessidade e a importância de conscientização da população local, torna-se necessário que sejam adotadas medidas preventivas, como por exemplo, correta gestão dos resíduos sólidos e líquidos, controle de vetores e pragas, qualidade apropriada da água e condições adequadas para produção, manipulação e consumo dos alimentos, assim como a capacitação e oferecimento de cursos junto á comunidade, para que sejam obtidas melhorias na qualidade e segurança dos alimentos vendidos bem como, nas condições de trabalhos dos próprios vendedores. Uma das formas para se atingir um alto padrão de qualidade e segurança em serviços de alimentação é a implantação de Boas Práticas.

3.4 Boas Práticas em Serviços de Alimentação

Boas práticas (BP) são definidas como uma série de procedimentos a serem realizados para atingir um determinado padrão de identidade e qualidade de um produto e/ou serviço na área de alimentos (STEDEFELDT et al., 2013). A finalidade das BP é diminuir as fontes de contaminação, durante a preparação dos alimentos, fornecendo, dessa forma, alimentos seguros ao consumidor (TONDO; BARTZ, 2011). São apresentadas abaixo algumas BP que devem ser aplicadas nos serviços de alimentação.

a) Edificação, instalações, equipamentos, móveis e utensílios

Muitos estabelecimentos comerciais no ramo de alimentação são edificados e instalados de forma incorreta e/ou em locais incorretos e, muitas vezes, arcaicos, adquiridos por proprietários sem conhecimento sobre a segurança de alimentos. As edificações e instalações devem ser projetadas de forma a

possibilitar um fluxo ordenado e sem cruzamentos nas etapas da preparação de alimentos, além de facilitar as operações de manutenção, limpeza e, quando for o caso, desinfecção (CODEX ALIMENTARIUS, 2006).

De acordo com a Portaria 78/2009, do Estado do Rio Grande do Sul, Brasil, os alimentos devem ser preparados e vendidos em um local limpo e bem iluminado, protegido do sol, poeira, chuva e vento. Devem igualmente, estar longe de fontes de contaminantes, como sólidos e líquidos, resíduos e de animais, incluindo os de estimação e pragas. As instalações utilizadas para preparação e vendas não devem ser um local, que possam levar à contaminação de alimentos com riscos biológicos, químicos ou físicos. A mesma Portaria considera que o piso e as paredes devem ser de material liso, impermeável e de fácil higienização e em adequado estado de conservação. Instalações sanitárias devem ser dotadas de lavatórios e supridas de produtos destinados à higiene pessoal (papel higiênico, sabonete líquido inodoro e antisséptico ou sabonete líquido inodoro e solução antisséptica, papel toalha não reciclado ou outro sistema higiênico e seguro de secagem de mãos). Os Coletores de lixo, nas instalações sanitárias, devem ser dotados de tampa acionada sem contato manual e devem ser higienizados sempre que necessário e, no mínimo, diariamente. Equipamentos, móveis e utensílios que entram em contato com alimentos preparados devem ter desenhos que permitam a higienização, e em estado de conservação adequados, devem ser elaborados com materiais que não transmitam substâncias tóxicas, odores e sabores aos alimentos (RIO GRANDE DO SUL, 2009).

b) Higienização de instalações, equipamentos, móveis e utensílios

O processo de higienização é dividido em duas etapas distintas, limpeza e sanitização ou desinfecção. A limpeza é um procedimento que tem como objetivo a remoção de resíduos orgânicos e minerais (sujidades) que estão aderidos às superfícies, enquanto a sanitização tem como objetivo eliminar ou reduzir os microrganismos patogênicos até níveis considerados seguros (STEDEFELDT et al., 2013).

A higienização de instalações, equipamentos, móveis e utensílios em serviços de alimentação é um fator muito importante para a inocuidade dos alimentos. Estes procedimentos evitam, por exemplo, a contaminação cruzada,

que pode ocorrer quando os microrganismos são transferidos de superfícies contaminadas (ex. paredes, equipamentos, utensílios ou mesmo manipuladores) para alimentos, podendo representar um risco para à saúde do consumidor (TONDO; BARTZ, 2011).

A RDC nº 216, de 15 setembro de 2004 (BRASIL, 2004) considera que as instalações, os equipamentos, os móveis e os utensílios devem ser mantidos em condições higiênico-sanitárias apropriadas. A área de preparação do alimento deve ser higienizada quantas vezes forem necessárias e imediatamente após o término do trabalho. Substâncias desodorantes em quaisquer das suas formas não devem ser utilizadas nas áreas de preparação e armazenamento dos alimentos. Os produtos saneantes utilizados devem estar regularizados pelo Ministério da Saúde e a diluição, o tempo de contato e modo de uso/aplicação devem obedecer às instruções recomendadas pelos fabricantes. Os produtos saneantes devem ser identificados e guardados em local reservado para essa finalidade. Os fornecedores de alimentos devem higienizar os utensílios entre o uso ou devem utilizar utensílios descartáveis (de preferência recicláveis ou biodegradáveis), sempre que possível. Quando necessário, os alimentos devem ser embrulhados em papel limpo, plástico ou outro material adequado para alimentos. Papel de jornal, papel usado e outros materiais de embalagem não higiênicos não devem ser usados em contato direto com alimentos (BRASIL, 2004; WHO, 1996). Práticas higiênicas eficientes são necessárias em todas as etapas de produção de alimentos, sendo por isso importante que os fornecedores empreguem procedimentos de limpeza que garantam que as unidades de venda, equipamentos e utensílios estejam devidamente limpos e higienizados (BANJUL; GENEVA, 2017).

c) Abastecimento de água

A água é uma matéria-prima crítica para a preparação de qualquer alimento, inclusive aqueles servidos em mercados e na rua. Ela também pode ser contaminada com perigos biológicos, químicos ou físicos. Como tal, a água contaminada pode configurar risco à saúde pública se usada para beber, utilizada na lavagem de alimentos, incorporada nos alimentos como

ingrediente, utilizada no processamento de alimentos ou usada na lavagem de equipamentos, utensílios e recipientes (WHO, 1996).

A legislação Moçambicana (Diploma Ministerial N° 51/84, de três de Outubro) assim como a Brasileira (BRASIL, 2004) recomendam somente a utilização de água potável para a preparação de alimentos em serviços de alimentação. Caso seja utilizada uma solução alternativa de abastecimento de água, a potabilidade deve ser atestada semestralmente mediante laudos laboratoriais (BRASIL, 2004; MOÇAMBIQUE, 1984; RIO GRANDE DO SUL, 2009).

A RDC N° 216/2004 (BRASIL, 2004) estabelece que o reservatório de água deve ser edificado de materiais que não comprometam a qualidade da água (livre de rachaduras, vazamentos, infiltrações, descascamentos e devidamente tampado), em adequado estado de higiene e conservação, devendo ser higienizado em intervalo de tempo máximo de seis meses. No caso da utilização do gelo para o consumo ou que entre em contato com alimento ou superfície, ele deve ser produzido com água potável, além de ser manipulado e estocado em condições higiênico-sanitárias satisfatórias (BRASIL, 2004).

A água usada para lavar utensílios, alimentos e mãos deve ser segura. A preparação dos alimentos deve ser acompanhada de uma correta higienização principalmente quando se trata de vegetais folhosos consumidos crus. A água potável corrente deve estar disponível para esses fins (BRASIL, 2004; RIO GRANDE DO SUL, 2009). Um dos problemas mais críticos na venda de comida de rua é o suprimento de água de qualidade aceitável e em quantidades suficientes para beber, lavar, limpar e outras operações. Os vendedores ambulantes podem transportar apenas suprimentos limitados até as barracas ou veículos de comida dentro dos mercados ou nas ruas, uma vez que esses serviços de alimentação, muitas vezes, não têm acesso direto ao abastecimento de água potável. De fato, a água é geralmente escassa em áreas de baixa renda em muitos países em desenvolvimento. As torneiras de água podem funcionar apenas por algumas horas durante o dia e, às vezes, não funcionam por dias.

Os vendedores ambulantes em várias partes do mundo são conhecidos por lavar seus utensílios, incluindo aqueles em que os alimentos foram servidos, na

água que foi usada anteriormente. A manutenção da qualidade da água durante a lavagem é de vital importância, pois ajuda a minimizar potencial contaminação microbiana da água de processamento e contaminação cruzada subsequente do produto (MIR et al., 2018). Portanto, o abastecimento de água precisa de muita atenção nas operações de comida de rua (WHO, 1996).

d) Manipuladores de alimentos

Considera-se manipulador de alimentos qualquer pessoa num serviço de alimentação que entra em contato (direto ou indireto) com o alimento (BRASIL, 2004). Eles muitas vezes são responsáveis pela ocorrência de surtos alimentares e/ou DTA porque cerca de 30 a 40% dos humanos são portadores assintomáticos de microrganismos. O corpo humano apresenta muitos microrganismos, principalmente em nível intestinal, então se os manipuladores não realizam procedimentos adequados de higiene, como por exemplo, a correta lavagem e antissepsia das mãos, podem contaminar os alimentos com microrganismos patogênicos (TONDO; BARTZ, 2011).

Além de serem portadores naturais e assintomáticos, os manipuladores de alimentos podem apresentar perigos biológicos, quando sofrem de doenças de pele, intestinais, ou quando os organismos do trato respiratório contaminam alimentos ou superfícies de contato com alimentos ou por contaminação cruzada, após o manuseio de matérias-primas. Perigos físicos também podem ser introduzidos por manipuladores de alimentos que usam jóias, ataduras ou práticas descuidadas de manipulação de alimentos (WHO, 1996).

Frequentemente as DTA são causadas por microrganismos como *Staphylococcus aureus*, bacilos gram negativos e vírus (hepatite A, Norovirus e outros) (DAS et al., 2017). Muitos desses patógenos podem ser naturalmente encontrados na mão, intestino, boca, pele e cabelo, por várias horas, dias ou anos, após a contaminação inicial. Quando não há observância das medidas básicas de higiene pessoal, as bactérias podem entrar em contato com os alimentos e em alguns casos, multiplicarem-se a ponto de causar doenças aos consumidores (ASSEFA et al., 2015).

A observância de regras de higiene pessoal por parte dos manipuladores de alimentos pode diminuir expressivamente a ocorrência de surtos e ou de DTA (ASSEFA et al., 2015). Portanto, os manipuladores de alimentos devem

lavar as mãos com água e detergente líquido inodoro e realizar a antissepsia, após se envolverem em quaisquer atividades suscetíveis de contaminação por perigos biológicos, químicos ou físicos (por exemplo, antes e após o manuseio alimentos crus de origem animal e vegetal, depois de usar o banheiro, depois de manusear objetos insalubres, como recipientes de lixo, após tocar em papelão, embalagens ou animais e após contato com substâncias químicas, como resíduos de pesticidas e desinfetantes).

Na preparação e venda de alimentos, os manipuladores devem abster-se de práticas anti-higiênicas e inadequadas, tais como mascar, fumar, falar, cantar, assobiar, espirrar, cuspir, tossir, manipular dinheiro ou praticar outros atos que possam comprometer a integridade do alimento durante o desempenho das suas atividades (RIO GRANDE DO SUL, 2009). Os manipuladores devem ter asseio pessoal, apresentando-se com uniformes limpos, conservados, e compatíveis às atividades, devendo ser trocados no mínimo diariamente e usados exclusivamente nos serviços de alimentação. Além disso, devem ter capacitações periódicas em temas sobre a segurança de alimentos, devendo essas capacitações serem comprovadas mediante a apresentação de documentação (BRASIL, 2004).

e) Registros

Muitos gestores/manipuladores não consideram importantes a documentação e registros de qualidade, contudo sem eles o estabelecimento não tem como fazer uma gestão da segurança de alimentos adequada ou análise correta do seu histórico, assim como não é possível comprovar a realização de um determinado procedimento realizado no passado. Por isso todas as operações, tais como higienização das instalações, equipamentos e móveis, controle integrado de vetores e pragas urbanas, higienização do reservatório de água, controle da higiene, saúde dos manipuladores capacitações, entre outras, devem ser registradas (BRASIL, 2004; TONDO; BARTZ, 2011).

f) Preparação do alimento (tempo e temperatura)

A cozinha, pela sua natureza (local úmido, presença de nutrientes e temperaturas elevadas) é considerada um ambiente propício para a contaminação e multiplicação de microrganismos, sendo por isso o binômio tempo e temperatura um dos fatores mais importantes a serem controlados, durante o processamento, manipulação e distribuição dos alimentos (DA SILVA JR, 2014). Por exemplo, é necessário que durante o tratamento térmico a temperatura mínima de 70°C (BRASIL, 2004) seja atingida em todas as partes do alimento. A cocção adequada pode eliminar boa parte da contaminação decorrente da matéria-prima (TONDO & BARTZ, 2019).

Após os alimentos serem submetidos à cocção eles devem ser distribuídos à temperatura mínima de 60°C, por um período máximo de seis horas, ou sob refrigeração. O tempo necessário para transportar alimentos entre as unidades de preparação e venda deve ser tal que a proliferação microbiana não atinja níveis perigosos nas condições de transporte e venda. Os problemas de transporte são minimizados se o ponto de venda estiver próximo do local de preparação (RIO GRANDE DO SUL, 2009; WHO, 1996). Alimentos preparados que devem ser servidos frios e que podem propiciar a multiplicação de patógenos, devem ser armazenados a temperaturas inferiores a 5°C e podem ser conservados durante cinco dias, enquanto alimentos congelados devem ser conservados em temperaturas iguais ou inferiores a -18°C (RIO GRANDE DO SUL, 2009; WHO, 1996).

Portanto, o controle do binômio tempo x temperatura mostra-se como um dos fatores mais importantes a ser controlado para manter a qualidade microbiológica e segurança dos alimentos.

3.5 Conhecimentos, atitudes e práticas dos manipuladores de alimentos

As DTA são de importância para à saúde pública em todo o mundo e grande parte dos fatores relacionados à sua ocorrência está sob a responsabilidade dos manipuladores de alimentos (HAMED; MOHAMMED, 2019). O Conhecimento, as Atitudes e as Práticas (CAP) dos manipuladores de alimentos são importantes para identificar ações prioritárias no planejamento do treinamento e garantia de melhorias não apenas nos métodos de

treinamento, mas também na aplicação em programas de capacitação. A avaliação do CAP pode ser um dos primeiros passos para entender o ponto de vista do manipulador de alimentos (ZANIN et al., 2017).

A atitude é um fator crucial que pode afetar o comportamento da segurança de alimentos e práticas dos manipuladores, contribuindo para a diminuição da incidência de doenças (ABDULLAH SANI; SIOW, 2014; AL-SHABIB; MOSILHEY; HUSAIN, 2016). Atitude é o principal elo entre conhecimento e as práticas dos manipuladores. Estudos demonstraram que trabalhadores que têm conhecimento o traduzem com mais facilidade em práticas adequadas se tiverem uma atitude positiva e vice-versa (ZANIN et al., 2017).

Vários estudos sobre CAP sobre segurança de alimentos têm sido realizados e publicados em diferentes partes do mundo. Woh et al. (2016) com o objetivo de avaliar o conhecimento sobre práticas de segurança e manuseio de alimentos entre manipuladores de alimentos, realizaram um estudo transversal com 383 manipuladores em três grandes cidades da Malásia. Os resultados demonstraram que, de uma forma geral, os entrevistados tinham um baixo nível de conhecimento sobre segurança de alimentos.

Onyeneho e Hedberg (2013) entrevistaram 145 chefes de cozinha e gerentes de restaurantes de Owerri, na Nigéria, a fim de avaliar seus conhecimentos sobre segurança de alimentos, tendo focado em aspectos como seus conhecimentos de percepção de risco, práticas de manuseio de alimentos, controle de temperatura, patógenos transmitidos por alimentos e higiene pessoal. Noventa e dois por cento relataram que fizeram a limpeza e higienização dos equipamentos e superfícies enquanto 37% revelou não possuir conhecimentos sobre práticas que previnem a contaminação cruzada. Associado a isso, quase a metade dos entrevistados (49%) relatou ter permitido que uma pessoa com algum tipo de infecção microbiológica manipulasse alimentos.

Al-Kandari et al. (2019a) avaliaram o nível de conhecimentos, atitudes e práticas em segurança de alimentos entre 402 manipuladores em restaurantes do Kuwait. Os resultados demonstraram que a maioria (70%) dos manipuladores de alimentos possuía conhecimento suficiente, tendo mostrando resultados muito bons particularmente sobre higiene pessoal (93%). Áreas

mais preocupantes foram à falta de conhecimento sobre a contaminação cruzada e saneamento (68%), controle de tempo e temperatura para alimentos (63%) e patógenos alimentares (51%).

Da Cunha et al. (2019) realizaram uma pesquisa com o objetivo de diferenciar entre as práticas de segurança de alimentos observadas e autorreferidas pelos manipuladores de alimentos, e concluíram que os manipuladores com alta percepção de risco sobre suas práticas relataram práticas menos adequadas e as atitudes reforçaram o efeito positivo entre o conhecimento e práticas observadas. Associado a isso, Zanin et al. (2017) avaliaram a relação entre CAP de alimentos manipuladores com treinamento em segurança de alimentos. O estudo destacou pouco conhecimento e não conformidade com as práticas de segurança de alimentos entre os participantes, não só como também a falta de tradução adequada do conhecimento em atitudes e/ou práticas vice-versa. tendi, precisa melhorar) Vários autores consideram que programas de educação e treinamento eficiente em segurança de alimentos devem ser implementados para afetar positivamente as atitudes e práticas dos manipuladores e melhorar seus conhecimentos e práticas em segurança de alimentos.

3.6 Legislações aplicadas á serviços de alimentação em Moçambique e no Brasil

Em Moçambique, o documento que normaliza o funcionamento dos serviços de alimentação (estabelecimentos de alimentação) é o Diploma Ministerial N° 51/84, de três (3) de Outubro conhecido também como Regulamento sobre os Requisitos Higiênicos dos Estabelecimentos Alimentares. Neste documento estão descritos todos os procedimentos considerados indispensáveis para a defesa da saúde pública. Em muitas cidades de Moçambique não há fiscalização, e, quando existe, geralmente não contempla as questões sócio-econômicas do manipulador e seus conhecimentos sobre higiene e saúde, mas simplesmente a ocupação do espaço urbano e a posse de licenças.

No Brasil, a legislação que rege as Boas Práticas dos serviços de alimentação é a RDC N° 216, publicada em 15 de setembro de 2004 pela Agência Nacional de Vigilância Sanitária (ANVISA) (BRASIL, 2004). Esta legislação é nacional e a partir dela foram elaboradas legislações estaduais como é o caso Portaria N° 78, publicada em 30 de janeiro de 2009 do Estado do Rio Grande do Sul (RS) (RIO GRANDE DO SUL, 2009). Ambos os documentos foram criados com o objetivo de estabelecer procedimentos de Boas Práticas para serviços de alimentação, a fim de garantir às condições higiênico-sanitárias do alimento preparado. Ambas as legislações foram elaboradas tendo em consideração os princípios enunciados no Codex Alimentarius e surgem pela necessidade de constante aperfeiçoamento das ações de controle sanitário na área de alimentos, visando à proteção da saúde da população (BRASIL, 2004).

A RDC n° 216/2004 e a Portaria N° 78/2009, estão divididas em seções que abordam questões relacionadas à: Edificações, instalações equipamentos, móveis e utensílios; Higienização de instalações, equipamentos, móveis e utensílios; Controle integrado de pragas; Abastecimento de água; Manejo de resíduos; Manipuladores; Matérias-primas ingredientes e embalagens; Preparação do alimento; Armazenamento e transporte do alimento preparado; Exposição ao consumo do alimento preparado; Documentação e registro e Responsabilidades (BRASIL, 2004; RIO GRANDE DO SUL, 2009).

Apesar de existirem no Brasil legislações bem claras e rígidas em termos de regras e critérios sobre BP em serviços de alimentação, são poucos os estabelecimentos que as adotam como uma prática diária na produção de alimentos devido a dificuldades relacionadas a questões financeiras assim como falta de profissionais conscientes e comprometidos (TONDO & BARTZ, 2019).

O Diploma Ministerial N° 51/84, de três (3) de Outubro está dividido em (onze) capítulos e por sua vez os capítulos são subdivididos em secções. O primeiro capítulo aborda as definições; o segundo é sobre a regularização dos estabelecimentos alimentares. O terceiro é sobre os requisitos mínimos obrigatórios para os estabelecimentos alimentares; o quarto fala sobre as normas higiénicas para o pessoal; o quinto é sobre os requisitos mínimos obrigatórios (RMO) para estabelecimentos alimentares de produção e

embalagem. O sexto capítulo apresenta os RMO para armazenamento dos produtos alimentares; o sétimo os RMO para estabelecimentos alimentares de consumo; o oitavo capítulo refere-se aos RMO para os estabelecimentos alimentares de venda; o nono apresenta os RMO para os meios de transporte; o décimo fala sobre o Pessoal sanitário com competência para inspeções e por fim o decimo primeiro capítulo é sobre as Disposições finais (MOÇAMBIQUE, 1984).

O Diploma Ministerial N° 51/84, de três (3) de Outubro, não é um documento recente e, por causa disso, alguns elementos considerados importantes atualmente não são considerados. Por exemplo, falta no regulamento orientações sobre a inspeção das matérias-primas, ingredientes e embalagens, controle da temperatura no recebimento das matérias-primas e ingredientes; procedimentos sobre a realização correta de descongelamento dos alimentos; tempo de exposição ao consumo do alimento preparado; registros das temperaturas dos equipamentos utilizados para esse fim; higienização dos vegetais folhosos consumidos crus, assim como o controle periódico da água utilizada para a preparação dos alimentos.

Pelos motivos apresentados, investigar as condições higiênico-sanitárias e a contaminação microbiológica de alimentos, manipuladores e utensílios utilizados na preparação de alimentos em mercados e nas ruas de Maputo, Moçambique, torna-se essencial para que se identifiquem possíveis falhas nos procedimentos de higienização ou contaminação cruzada, e também auxiliar durante a preparação de programas de treinamento e desenho de planos estratégicos pelas entidades competentes.

4. RESULTADOS

A apresentação dos resultados dessa Tese de Doutorado foi dividida em quatro artigos. Cada seção deste capítulo corresponde a uma dessas publicações científicas. O artigo um contempla uma avaliação das boas práticas nos mercados da cidade de Maputo e o desenvolvimento do Índice de Segurança de Alimentos (FSI) dos estabelecimentos neles presentes. Este artigo foi publicado na revista *African Journal of Food Science*.

No artigo dois, submetido à revista *Journal of Infection in Developing Countries,* são apresentados os resultados da avaliação dos conhecimentos e atitudes sobre a segurança dos alimentos e investigação de *Enterobacteriaceae* nas mãos dos manipuladores e saladas de alface vendidas nas ruas da cidade de Maputo.

O artigo três, que foi publicado na revista *Journal of Food Nutrition Research*, corresponde a determinação de prevalência de *Enterobacteriaceae* em saladas prontas para consumo, água potável, mãos dos manipuladores e superfícies (bacias e facas) nos mercados da cidade de Maputo.

Por fim, o artigo quatro é um estudo preliminar sobre a prevalência de *Escherichia coli* em alfaces (*Lactuca sativa*) e saladas de alface prontas para o consumo, vendidas nos mercados da cidade de Maputo. Este artigo será submetido na revista na revista *Journal of Food Safety*.

4.1 Artigo 1

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Assessments of good hygienic practice in food markets of Maputo, Mozambique and development of Food Safety Index

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This study evaluated good hygienic practices (GHP) of food establishments in seven food markets in Maputo, Mozambique. A total of 191 food handlers were selected in this study. Information on demographic characteristics and hygienic-sanitary conditions were obtained through semi structured interviews. The results revealed that the majority of food vendors in Maputo are females (92.7%) and all the selected vendors (100%) have not been trained in food handling. Vendors had no access to ablution facilities and in instances where they are available, they are few and located far away from the area where food is prepared. In terms of storage, 91.1% of the food handlers kept their cooked food unrefrigerated with only 9.9% of the vendors having freezers. Of the 191 establishments, 85.76% had Food Safety Index (FSI) between 0.15 and 0.18 scale, being therefore classified as bad. Good hygienic practices are therefore necessary to improve market conditions and consequently prevent foodborne diseases. The recommendations from this study is to properly conduct food safety training for all food vendors improving vending infrastructure and implementing food safety inspections in the food markets of Maputo City.

Key words: Good hygienic practices, food handlers, food establishment.

INTRODUCTION

In recent years, there has been an increase in the number of people consuming food prepared by food vendors partly due to lifestyle changes influenced by globalization (WHO, 2015a). Eating out has provided a quick and practical solution for people who work and live in big cities. Public food vending markets in big cities usually have several kinds of food services. These food services play an essential role in the survival of medium and low-income earners in many countries, because they are cheap and easily accessible (McKay et al., 2016).

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1. Introduction

In recent years, there has been an increase in the number of people consuming food prepared by food vendors partly due to lifestyle changes influenced by globalization (WHO, 2015a). Eating out has provided a quick and practical solution for people who work and live in big cities. Public food vending markets in big cities usually have several kinds of food services. These food services play an essential role in the survival of medium and low-income earners in many countries, because they are cheap and easily accessible (McKay et al., 2016). Despite the advantages of food vending to the public, it represents a significant risk to public health due to their potential to spread pathogenic bacteria that can cause food-borne diseases (Kubheka et al., 2001).

The World Health Organization (WHO, 2015b) estimated that 600 million people in the world (one in 10 people) suffer from foodborne associated illness and become ill after eating contaminated food and about 420,000 of those affected die every year. Among the deaths caused by food-borne diseases, 125,000 are children under five years old (WHO, 2015b). In the United States, about 48 million people get ill, 128,000 are hospitalized and about 3,000 die each year due to food-borne diseases (FDA, 2019). The incidences are still high in developing countries; as more than 91 million people fall ill every year and 137,000 die due to food-borne diseases in Africa (WHO, 2015a).

Food services generally include catering, schools, and hotel kitchens, restaurants, and street vendors who usually serve ready-to-eat food to the public. Data on the occurrences and incidences of food-borne diseases in Mozambique is limited and is starting to accumulate. Recently, Macaza (2017), conducted a study in Nampula, Mozambique, and found out that 83% of the food samples collected were unsatisfactory due to high counts of Enterobacteriaceae like Escherichia coli. The hygienic conditions of foods vended in Maputo is unknown although it is of public record that the conditions of food services inside the public markets are not ideal. Therefore, the aim of this study was to evaluate the Good Hygienic Practices (GHP) and sanitary conditions in seven food markets in Maputo city, and from the results, identify priority control measures to avoid the outbreaks of food-borne diseases.

2. Materials and Methods

2.1 Study area design

This study was conducted in seven food markets located in Maputo city from January to March 2017. Maputo is the capital of Mozambique, a country with 28.83 million inhabitants. Maputo has more than 1.2 million inhabitants who reside in seven urban administrative districts, and these districts combined have a total of 12 food markets. The seven sampled food markets (Museu, Povo, Mandela, Estrela Vermelha, Benfica, Xipamanine and Peixe) were selected because they are the most visited food markets. Food establishments in markets are called “barracas” (stalls). These establishments are described as small food services or restaurants, where the general public goes to have breakfast and lunch. The foods in these establishments are generally adapted to the conditions of urban life and are prepared for the preferences of public market consumers. Typically, the food preparations involve products like rice and meat, accompanied by lettuce, tomato, and cabbage salads.

2.2 Sampling size

A total number of 191 food establishments were randomly selected and sampled from seven food markets in Maputo City. Food handlers and establishments were selected randomly from the roster list of food handlers, which was provided by market authorities. The markets were chosen based on the number of vendors operating, so the studied markets had a minimum number of 20 food vendors. Vendors who did not have contact with food were excluded from the interviews.

2.3 Data collection: tools and procedures

A semi structured questionnaire and secondary sources were used for data collection in this study. This method of data collection was chosen because it is easy to use and can be very efficient to obtain information in a short time. The legal basis for the construction of the questionnaire checklist was the Ministerial Diploma n. 51/84 of October 3rd, 1984, used in Mozambique with some modifications.

The check-list was structured into two different sections. Section one was designed to collect information on respondent’s socio-demographics such as

gender, age, level of education, and marital status. Part two was designed to collect information about GHP and sanitary conditions in the food services of each market.

2.4 Data quality control

The quality of data was assured by pre-testing of the questionnaire. The data collectors were trained on the objective, the importance of the study, the confidentiality of information, respondent's right, techniques of interview, and inspection of food services. Before the review, twenty food services were visited to pilot test the questionnaires, ensure their validity and reliability of information. Feedbacks from the data collectors were considered and the checklist was improved and approved. Senior investigators ensured the completeness and reliability of the information gathered throughout the data collection process.

2.5 Secondary information on microbial data

Microbial data of previously food surfaces, water, hands of food handlers and food sold in the local markets of Maputo was obtained from reference laboratory of Maputo City Health Directorate and presented in this study.

2.6 Food Safety Index

To analyze the hygienic-sanitary conditions of the establishments visited in the markets, a Food Safety Index (FSI, Equation 1) was created based on the work of Elias et al. (2015). This index was based on the calculation of the harmonic mean and considers different weights (Table 1) for each group of checklist issues. The presence of potable water and sewage issues were assigned with a weight of 16, considering the importance of these items for public health and food safety. Establishments without potable water and sewage present a very high possibility of causing foodborne diseases.

Questions about the training received weight 8, because without proper training, any control measures could not be by food handlers or vendors. Temperature control weight 6, cross-contamination weight 4, indirect contamination weight 2, and documents importance 1. These weights were based on Da Cunha et al. (2014a) and Elias et al. (2015).

$$FSI = \frac{\sum_{i=1}^{N} w_i}{\sum_{i=1}^{N} \frac{w_i}{x_i + 1}} - 1 \quad (1)$$

where xi = response of i question of the questionnaire, Wi = weight of i question, N = number of questions.

2.7 Data processing and analysis

After the interviews, the checklists had been reviewed for completeness. The items were coded and analyzed using SPSS version 25.0. The results were presented in tables and texts using descriptive statistics such as mean, standard deviation, and percentage, to describe the study population with relevant variables. The degree of association between independent and dependent variables had been assessed with a 95% confidence interval, and a level of significance of 0.05 had been used in research. No questionnaire was invalidated because all inquiries had been conducted by the author and a trained team of reviewers. Additionally, FSI was used to classify food establishments into three categories (Table 2).

2.8 Ethical consideration

Ethical approval and clearance was obtained from the Institutional Review Board of the Federal University of Rio Grande do Sul. Permission was also obtained from the administration health office and the City Council of Maputo city. Verbal consent obtained from officials responsible for the markets. Each respondent was assured that the information provided by her/him would be kept confidential and used only for the purposes of this research.

3. Results and discussion

3.1 Demographic factors

A total of 191 food handlers from seven food markets in Maputo responded to the checklist. The results demonstrated that food handlers in food market establishments were mostly single young women, with ages ranged from 20 to 30, and incomplete secondary education level (Table 3). This finding was consistent with studies in several parts of the world, such as Brazil (Da Cunha et al., 2014a, b; Rossi et al., 2017), Vietnãm (Samapundo et al., 2016),

Haiti (Samapundo et al., 2015), and Nigeria (Aluko et al., 2014). According to Allen and Sachs (2007), women are generally responsible for cooking meals worldwide. Furthermore, those ages from 20 to 30 already stop their education and use to cook to sustain their families or to complement ordinary income. According to Grant and Unacla (2012), the sale of ready-to-eat foods in public establishments is an activity that does not require training or significant investments. And it provides work for people who might be unemployed, including women living in urban areas. These findings explain the profile of food vendors in the markets in Maputo.

3.2 Characterization of sewage and water used for food preparation in markets

The finding demonstrated that all markets visited have access to drinking water provided by the public water system government (Table 4). It is a positive development because access to clean water is limited for millions of people around the world, particularly for the countries of Africa (WHO, 2011). Although markets are supplied with potable water, there is only one water collection point for all the markets. Therefore, the establishments do not have piped water, which forces sellers to transport and store water in containers that are rarely sanitized (visually dirty). The lack of piped water reduces the possibility for handlers to perform the procedures of cleaning hands, cleaning, and sanitizing equipment which greatly compromise food safety. The microbiological quality and safety of the food prepared using water that was stored in the establishments is not assessed. However, water from the single distribution point at each market, before the water was stored in the plastic containers of each food service, is periodically tested. Mozambique's regulations do not require periodic monitoring of the water used in food facilities as required by other legislation of other countries like Brazil 2004; Rio Grande do Sul, 2009.

Wright et al. (2004) reported that water analysis performed only at the general source of supply might not reflect the quality of water used for food preparation. Handlers can contaminate water with their own hands or through containers used for storage. This inadequate situation is not unique to Mozambican public markets, since globally, at least 1.8 billion people use a drinking water source contaminated with feces (WHO, 2019).

Among the seven markets visited, only Peixe market was equipped with excellent sewage facilities and adequate facilities for disposal of waste during the peak discharge period (Table 4), as outlined in Ministerial Diploma no. 51/84 of October 3rd (Mozambique, 1984). Lack of adequate waste disposal systems contributes to the increase in the number of diarrheal diseases resulting from inadequate sanitation of the environment (WHO, 2015b). Lack of sanitation can force people to defecate in the open, posing a public health hazard and increasing the possibility of contamination of the environment and foods (WHO, 2018). Improving water supply and sanitation can help economic growth in low- and middle-income countries, contributing to poverty reduction (WHO, 2019). Therefore, investments in infrastructure that improve basic hygiene and ensure safe drinking water should be a priority.

3.3 Training

The results of this research demonstrated that all food handlers (100%) in the study have never been trained in food safety issues (Table 4). This situation was considered inadequate since adequate food safety training for handlers is very important to promote Good Hygienic Practices during food preparation. Education and training a fundamental principle of food safety, as they contribute to the reduction of foodborne disease cases (Hassan et al., 2018; Moreb et al., 2017; Osaili et al., 2013). Da Cunha et al. (2014b), investigated the influence of training in food safety and reported that food handlers who received training had higher scores on food safety knowledge. In addition, Soon et al. (2012), evaluated the knowledge about food safety in food handlers in institutional food services and concluded that trained food handlers demonstrate greater awareness of safe food preparation. Therefore, handlers' training programs in the markets of the city of Maputo in particular and in other parts of the world is essential to promote safe food handling practices in food preparation and sales. A study carried out in Portugal, aimed to evaluate the influence of food safety training in total plate counts of foods, demonstrated that instructions contributed to decreasing about 60% of microbiological counts (Soares et al., 2013).

3.4 Temperature control

In the present study, none of the establishments kept cold foods ready for consumption at less than 5°C (Table 5). Also, none had separate refrigeration and freezing equipment as outlined in Ministerial Diploma no. 51/84 of October 3rd. On the other hand, hot ready-to-eat foods were stored at temperatures above 60°C in 96.3% of the establishments. This practice keeps food safe because pathogenic bacteria do not multiply at temperatures above 60°C, and many of them also are inactivated. Food temperature control is one of the most important factors to be observed to maintain the microbiological safety of food. During the research, we also sought to know the destination of food leftovers since the markets do not have equipment for food preservation. The food handlers responded that they would take them home, keeping them at room temperature until consumption, at dinner. This practice represents a risk to sellers' families. Several studies have already shown that keeping food cooked for more than 2 h at room temperature may be responsible for food borne diseases outbreaks (FDA/NSTA, 2008; Hassan et al., 2018; USDA, 2013).

Regarding the freezing of foods at temperatures below 0°C, only 12.6% (n = 23) responded that they adopt this practice. Freezing does not kill microorganisms but inhibits their multiplication by keeping food safe for long periods (Chattopadhyay and Adhikari, 2014; Harrison et al., 2013; Ojha et al., 2016; Tang and Lum, 2015). These results explained by the fact that most markets do not have freezers for food preservation. Most markets in the city of Maputo did not have a minimum infrastructure, and even electricity was lacking in the establishments. Most markets in the city of Maputo consisted of tents or benches made of precarious material (leftovers of zinc and other materials) and most of them without firm cover. These establishments (pews) were made using parts of tree trunks or zinc plate leftovers even though these materials do not offer much security to the structure of the establishments. About the existence of cold equipment in the markets for food preservation, it was possible to verify that only (n = 23) establishments kept frozen foods. FAO (2016), considers that in low-income countries, such as Mozambique, cooling facilities, and infrastructures for the storage of food is scarce. The lack of cold equipment in the markets visited can also be associated with the small number of meals

served daily by each vendor (on average, 23 meals/day). So food is bought and prepared on the same day, with no need for extended storage.

3.5 Cross-contamination

For the evaluation of this item, ten questions were asked to the food handlers (Table 6). Results from this study showed that most handlers (91%) did not wash their hands properly before beginning to work on food preparation or after using the toilets.

Hand washing is an essential measure to prevent the spread of foodborne diseases and is considered one of the primary practices that reduce the transfer of bacteria from person to food (Lambrechts et al., 2014; Sibanyoni et al., 2017). Several studies have demonstrated that the non-hygienic habits are responsible for many food bone disease outbreak (Lambrechts et al., 2014; Nasrolahei et al., 2017; Todd at al., 2007). A study carried out in Nigeria to evaluate the presence of microorganisms in hand washing water from 246 people detected several food borne micro-organisms like Staphylococcus aureus, Escherichia coli, Enterobacter species, and Shigella species (Chinakwe et al., 2012). Understandably, handlers in the markets of the city of Maputo do not have a habit of washing their hands during food handling. Similar results in which most manipulators do not have the habit of washing hands during food handling was reported in Brazil and Ghana, were only 23 and 20%, respectively demonstrated have this practices (Alves da Silva et al., 2014).

Furthermore, the results from this study show that only a part of the food handlers (28.3%) presented clean, with nails cut, hair, and beard in conditions of cleanliness. It had also been observed that usually, the manipulators (67.0%) do not adopt practices (like no smoking, talking, coughing, and manipulating money). This situation compromises the safety of the food sold in these places (Codex Alimentarius, 2006).

During the present research, we observed that in most markets, the slaughter and evisceration of small animals, such as the chicken, was carried out in the same place where meal is prepared. It puts to risk the food safety because these animals are reservoirs of pathogenic microorganisms such as Salmonella and Campylobacter leading to possible cross-contamination.

Only 19.9% of establishments visited have an adequate layout. Inadequate design can promote food cross-contamination in food establishments. The problem of cross-contamination in food establishments does not only occur in countries with weak structures, such as Mozambique. Djekic et al. (2014) researched three European cities and concluded that inadequate layout and lack of space for the workers', can be several cases of cross-contamination in establishments. Based on this, the Maputo city government must improve market infrastructures to reduce the risks of cross-contamination, minimizing the occurrence of foodborne diseases related to food consumption outside the home.

3.6 Indirect contamination

In the markets of Maputo city, the vendors use coal as a source of energy for food preparation, which can be a source of physical and chemical contamination for food. Soot from the use of fuel causes dark stains on the walls and clothes of the handlers, making them difficult to sanitize. Codex Alimentarius (2006), recommends that food establishments must have bright flooring and walls that are easily sanitized to reduce the risk of contamination in food. Among the establishments analyzed, only 28.3% showed compliance with this item. Similar results, in which most food establishments did not have easily sanitized floors and walls, were found by Adikari et al. (2016) in food establishments in Sri Lanka. A possible microorganism present in food processing environments is Listeria monocytogenes. The bacteria are pathogen that can cause a high mortality rate for groups at risk. L. monocytogenes is capable of forming biofilms, which are quite tricky to remove and can be found in equipment and utensils, floors and drains, and can contaminate food through cross-contamination (Donlan, 2002; De Souza et al., 2014; Dzieciol et al., 2016).

The markets do not have private sanitary facilities for workers, do not have individual closets for employees, do not have detergents and alcohol for hand hygiene and have no adequate clean facilities for the number of users (Table 7). In the establishments, there is no proper plan or action for controlling flies, insects and rodents which may cause indirect contamination. Curtis et al. (2000) reported that insects and rodents should not have access to storage and

consumption areas, because they are reservoirs of enteric pathogens such as E. coli, Salmonella spp., and Campylobacter jejune were detected on surfaces used for food preparation as well as in water reservoirs.

Regarding trash management, only 10.5% of the establishments have an efficient system for the removal of garbage from the handling areas, and 26.2% store garbage in places far from the handling areas.

3.7 Documents

All food establishments did not have written guidelines for food handlers on proper hygiene and other hygiene habits. Only 42.9% had a sanitary license to perform the service (Table 8). A study done in Brazil, in 2013 concluded that the items related to documentation and safety registers are generally deficient in food establishments (Saccol et al., 2013), results that corroborate with the present study.

The fact that the food services are producing foods even without a sanitary license is a legal problem that can also reflect in food safety because sanitary officers use to check if the food establishments have the minimal conditions to produce food. However, even having a sanitary license, there is the possibility of food contamination by the environment, equipment, and utensils, and if handlers do not accomplish the Good Hygienic Practices (GHP). For example, a study carried out in Kenya-Nairobi revealed the presence of Entamoeba histolytica and Giardia lamblia on the hands of workers at certified establishments (Kamau et al., 2012). Thus, inspections are an essential part of the process to prevent foodborne illness, but the best way to protect the consumer is to prevent contamination by proper implementation of GHP (DeWaal, 2007).

3.8 Secondary information on microbial data

Microbial data from the reference laboratory carried out by the city health showed that the maximum and minimum values of total coliforms obtained in all samples were between 4.38 ± 0.58 and 0.1 ± 0.31 log CFU/ml. Water from the general deposit did not present any significant contamination. However, water from the containers of the establishments was contaminated by total coliforms (0.58 ± 0.63 CFU/ml), an indication of the lack of hygiene in food preparation.

As shown in Table 11, hands and utensils did not contain E. coli counts. Also, utensils did not reveal the presence of Staphylococcus aureus. However, total and fecal coliforms were found in both type samples. This contamination may have its origin from the water used for the preparation of food, but also from food handlers. It is known that the manipulator's hands are a potential way for bacteria pathogenic transmission and may be a possible cause for outbreaks (Assefa et al., 2015).

3.9 Food Safety Index (FSI)

In order to analyze the level of conformity of the establishments visited in the markets, FSI had been calculated. Table 2 presents the frequency of the distributed index into three categories: bad condition, satisfactory condition, and good condition. It is possible to state in a general way that the establishments (stalls) in the markets presented a very low FSI, an average of 0.18. The market stalls of Mandela and Xipamanine were those with minimum FSI values (0.15), and the Peixe market stalls showed maximum values of FSI (0.44) (Table 9). So we can say that out of a total of 191 stalls in the seven food markets visited, none got rated as in good conditions. In addition, 86% (n = 6) scored in bad condition and only one, 14% (n = 1) scored satisfactory condition (Table 10). The Peixe market was the one that presented an average rating. This market, compared to the others, presented better scores on the issues related to the exposure of food, practices that prevent cross-contamination and issues related to cross contamination. This situation is because this market benefited from rehabilitation one is the reference in the sale of shellfish and to be a tourist point.

The remaining markets obtained low FSI due to the lack of procedures like control of temperature, high exposure time after food preparation, lack of actions to prevent pests on the premises, lack of knowledge on the origin of water used for preparation of ice and also the sewers were not in right conditions, do not have an adequate disposal system, the establishments are not paved and not made of natural sanitizing material.

3.10 Association of socio-demographic and good practice variables

Socio-demographic variables were statistically tested for association according to the good practice procedures, using the Chi-square test with a significance level of 5%. Age and gender did not generally affect proper practice procedures in food outlets. Stratev et al. (2017) also noted that there was no significant difference ($p>0.05$) on food safety practices among students based on their ages. There was only a significant difference between the level of schooling and the habit of carrying out hygienic operations at the facilities (Pearson's Chi-square $p = 0.043$). It was evident that the more education the interviewed people had, the more care was taken to clean the facilities. Kennedy et al. (2011), suggested that the lack of knowledge of young people about the principles of domestic hygiene is justifiable by the fact that they are generally less involved in domestic activities, so, it seems essential to educate them from an early age so to stimulate GHP (Taché and Carpentier, 2014).

The Peixe market stood out to be the best of all the other markets studied (Table 5). There were also differences between markets. Peixe Market and Museu food handlers which had the habit of not smoking, talking, whistling or manipulating money during food manipulation ($p = 0.03$), and even concerning the floors of establishments that was easily sanitized ($p <0.05$). So we can conclude that there were significant differences ($p<0.05$) in markets and procedures on good practices.

Taking into account the priorities of the markets that presented a low FSI which allowed classifying them in markets of bad condition, we can suggest the control of water as a priority concerning food and ice preparation. It is necessary to ensure that the water used in the markets visited is free of contamination after its deposit in barrels, and this can be done employing the hygiene of the containers.

The second priority is to improve the infrastructure conditions of the markets. There must have facilities that allow vendors to have refrigerators to conserve leftover food so that they can be eaten safely in their homes. Improved facilities will also allow vendors to use other sources of energy to allow the walls and floors of the facilities to be easily rinsed and sanitized.

Analyzing the actions of salespeople, we can consider training as the third priority. Training vendors of food can be able to recognize the importance of hand hygiene before handling food.

4. Conclusion

The hygienic-sanitary conditions of food establishments in the public markets of Maputo were evaluated in this study. The public consuming food from food markets are at risk of food borne diseases because the hygienic-sanitary conditions of food preparation in Maputo city markets do not comply with the minimum requirements of the Mozambican legislation, as well as international law. There is a need for periodic inspections by local authorities to verify the conformity of minimum requirements for food safety. Implementation of training programs for food handlers, emphasizing the need for the application of GHP is also important to minimize foodborne diseases' risk.

In the priorities of the markets, the use of drinking water in all food services for food preparation is a short-term priority. It should be followed (in the medium term) by improving the infrastructure conditions of the markets. So the vendor can use equipment like a refrigerator and freezers for food preservation and also for the use of other energy sources such as domestic gas (liquefied petroleum gas) for food preparation. The third priority would be training food handlers on personal hygiene and GHP in food services. In the long term, the Mozambican government promotes safe food handling by integrating food safety into national policies and programs through several communication ways. Also, it is vital that the government prioritizes food safety and public health by ensuring that food suppliers act responsibly and provide safe food to consumers.

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7. Conflicts of interest

The authors declare that there are no conflicts of interests.

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Table 1
Weights of the weighted Food safety Index questionnaire

Parameterj	Weight
Water and sewage	16
Training	8
Temperature control	6
Cross contamination	4
Indirect contamination	2
Documents	1

Table 2
Classification according to the level of conformity of the weighting Food Safety Index (FSI)

FSI	Conformity level
0-35	Bad condition
36-75	Satisfactory condition
76-100	Good condition

Table 3
Demographic characteristics of respondents (n = 191)

Socio-demographic Characteristics	Frequency	Category	Percentage
Gender	14	Male	7.3
	177	Female	92.7
Age (years)	6	≤ 20	3.1
	68	21 – 30	35.6
	61	31 - 40	31.9
	33	41 - 50	17,3
	20	51 - 60	10.5
	3	> 60	1.6
Educational Level	84	Primary school	44
	99	Secondary	51.8
	3	University	1.6
	5	No qualification	2.6
Marital status	55	Married (a)	28.8
	88	Single	46.1
	39	Union fact	20.4
	2	Divorced	1.0
	7	Widower	3.7

Table 4
Answers to the questions about water, sewage and training

Questions	Response % Yes	No
Does the establishment have available potable water?	100	0.0
Are the sewers in good condition with watertight ducts able to guarantee complete disposal of waste during the maximum discharge period?	11.0	89.0
Is it ensured frequent minimum hygiene care training to the workers?	0.0	100.0

Table 5

Answers to the questions about temperature control

Questions	Response % Yes	No
Are perishable cooked foods that are not kept warm, kept at a maximum temperature of 4°C?	0.0	100.0
Are frozen foods stored below 0°C?	12.6	87.4
Are cooked foods that are kept warm stored at a temperature of not less than 60°C?	96.3	3.7
Does the establishment have refrigerators, and distinct freezers for each product nature, in order to ensure the ideal temperature?	8.8	91.2
Is the origin of the ice production water known?	25.1	57.1

Table 6

Answers to the questions about cross-contamination

Questions	Response % Yes	No
Do workers who present one of these symptoms / illnesses (skin disease, infected wounds, diarrhoea, hepatitis, tonsillitis, and tuberculosis) are put away from food preparation?	41.9	58.1
Do the food handlers wash their hands immediately before work, after using the lavatory, and whenever necessary, with potable water and appropriate detergent?	9.9	90.1
Are the toilets equipped with sufficient running water, washbasins with soap and whenever possible with a hand drying system?	9.9	90.1
Are food handlers clean, with fingernails cut, hair and beard clean, and wear no jewelry?	28.3	71.7
Are food intended for consumption without prior washing, cooking or peeling and that have no protecting package, taken with tweezers or other appropriate instruments, avoiding hand contact ?	67.0	33.0
Are the dishes and cutlery used by customers thoroughly cleaned with hot soapy water immediately after use?	0.0	100.0
Do workers have the habit of not smoking, talking, whistling, coughing, eating, manipulating money or practicing other acts that could contaminate food?	23.4	76.6
Do the kitchens have different sectors for: preparation of vegetables, animal origin food preparation and cooking?	0.0	100.0
Does the kitchen have separate refrigerators for the conservation of fruits and vegetables and other deteriorating foods that guarantee the temperature as established?	0.0	100.0

Table 7

Answers to the questions about indirect contamination

Question	Response %	
	Yes	No
Adequate waste disposal system	10.5	89.5
Are containers used for frequent garbage disposal kept away from production sites in a protected area?	26.2	73.8
Do the toilets have no direct communication with the places of storage, processing, packaging, consumption or sale of food products?	100.0	0.0
Do workers' sanitary facilities have locker rooms with washable, disinfectable cabinets, or individual hangers with a wardrobe for personal clothing and work uniforms?	0.0	100.0
Are the facilities, equipment and utensils kept in perfect hygienic condition with daily cleaning operations?	33.5	66.5
Control and prevention of insects, rodents and other parasites,	0.0	100.0
Are there domestic animals and obsolete objects in the food preparation area?	22.0	78.0
Does the food establishment have washable material floor in places where food is stored, prepared or served?	41.3	58.7
Has the facility been designed, constructed and arranged to allow fast, thorough cleaning and to prevent the penetration of birds, rodents and insects?	19.9	80.1
Is the establishment/Market located in a health and safety area free from smoke, unpleasant odors, dust and that is not subjected to flooding?	69.2	30.8
Do the establishments have no communication with housing?	100.0	0.0
Are the toilets paved, waterproof, washable and sanitizable?	100.0	0.0
Is the number of toilets as indicated in the regulation? (1 toilet for 9 workers, 2 toilets for 10 to 24 workers, 3 toilets for 25 to 49 workers)?	0.0	100.0
Are there showers in number that satisfies the type of the establishment and that corresponds to the number of workers?	0.0	100.0
Are there sanitary facilities only for workers?	0.0	100.0
Do the establishments have hygienic services available to the public, in adequate numbers for the establishment capacity?	0.0	100.0
Does the establishment have separate locations for kitchen, warehouse and dining room?	16.2	83.8
Does the kitchen have waterproof washable walls up to 1.80 meters high?	25.7	74.3

Table 8

Answers to the questions about documents

Question	Response %	
	Yes	No
Is there in the lavatory sector, a clearly written notice and drawings indicating the obligation to wash the hands after using the toilet?	0.0	100.0
Does the establishments have a sanitary license to exert the activity	40.3	59.7

Table 9

Classification of food markets based on the FSI (Food Safety Index) in Maputo City

Name of Market	Average	Category
Peixe	0.44	Medium
Estrela vermelha	0.16	
Xipamanine	0.15	
Mandela	0.15	Low
Povo	0.18	
Museu	0.18	
Benfica	0.17	

Table 10

Frequency distribution of values of weighted Food Safety Index according to low, medium and high level.

FSI	N	%
Low	6	85.7 ~ 86.0
Medium	1	14.3 ~ 14.0
High	0	0

Table 11

A list of all the food samples tested and quantification microorganisms analyzed

Type of sample	Performed analysis	Results (CFU)/ml ou g
Network Water (general deposit)	Total coliforms	(n=20) 0.0
Water from market Reservoirs	Total coliforms	(n= 15) (n=3)0.58± 0.63 UFC/ml
Hands	*Escherichia coli*	(n=10) 0.0 UFC/ml
	Total coliforms	(n=10) 0.52±0.62 CFU/ml
	Fecal coliforms	(n=10) 0.27±0.57 CFU/ml
	Staphylococcus coagulase positive	(n=10) 0.1±0.31 CFU/ml
Utensils	*Escherichia coli*	(n=10) 0.0
	Total coliforms	(n=10) 0.80±0.66 CFU /ml
	Fecal coliforms	(n=10) 0.53±0.58 CFU /ml
	Staphylococcus coagulase positive	(n=10) 0.0
Vegetables and e Salads	Total coliforms	(n=10) 4.38±0.58 CFU /ml
	Total coliforms	(n=10) 3.27±0.58 CFU /ml
Rice	Fecal coliforms	(n=10) 0.0
	Staphylococcuscogulaseposetive	(n=10) 0.0
	Bacillus cereus	(n=10) 0.0
Curry	*Escherichia coli*	(n=5) 0.0
	Total coliforms	(n=5) 0.0
	Fecal coliforms	(n=5) 0.0
	sulfite-reducing Clostridium	(n=5) 0.0
Soups	Total coliforms	(n=10) 0.0
	Fecal coliforms	(n=10) 0.0
	Bacillus cereus	(n=10) 0.0
	sulfite-reducing Clostridium	(n=10) 0.0

4.2 Artigo 2

Artigo a ser submetido à revista *Journal of Infection in Developing Countries*

Food safety knowledge, and attitudes and investigating *Enterobacteriaceae* on hands of food handlers and ready-to-eat vegetable salads vended in streets of Maputo City, Mozambique

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Abstract

Street-vended foods are important in providing urban dwellers with affordable and accessible food. However, poor hygiene of street-vended foods may contribute to foodborne diseases. This study evaluated the food safety knowledge and attitudes of food handlers and investigated *Enterobacteriaceae* on hands of food handlers and ready-to-eat salads vended in Maputo City,

southern Mozambique. A total of 110 vendors, selling ready-to-eat salads were randomly selected at various street-food vending sites in Maputo municipality. A questionnaire was used to collect data on the demographic characteristics, food safety knowledge and attitudes of the street vendors in face-to-face interviews. Ready-to-eat salads and hand samples were collected and analyzed using the ISO 21528-2 standard method for *Enterobacteriaceae* and isolated microorganisms were further identified by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). The majorities of interviewed people were females, younger than 35 years of age and have never received food safety training. A large percentage (42%) of the food handlers had insufficient knowledge about food safety, especially regarding cross-contamination, temperature control and pathogens. In contrast, most of the vendors had adequate knowledge about personal hygiene practices including the necessity of washing hands before starting to prepare foods, after using the toilet, after touching garbage and raw meat. The mean count of *Enterobacteriaceae* in ready-to-eat salad and on hands of vendors were 4.23 ± 0.78 log cfu/g and 3.14 ± 0.7423 log cfu/g, respectively. Generic *Escherichia coli* were detected in only one hand sample. No significant positive correlation ($P < 0.05$) was observed between the investigated knowledge and attitudes and results about indicator microorganisms. More research is needed to increase the understanding about this lack of correlation.

Keywords: Foodborne disease, street food vendor, food safety, microbiological quality

1. Introduction

Street-vended food is a popular choice among low and middle income consumers as it is cheap, nutritious, convenient and easily accessible [1–3]. Street vendors are an integral part of urban economies worldwide, especially in many developing countries where the choice of food is not varied [4, 5]. However, street food vending is a common practice in the informal market sector, are generally located in inappropriate places where there are no bathrooms or adequate facilities for washing hands and close to open drains as well rubbish bins [5]. Therefore, food products selling on the street are constantly exposed to potential contamination that can represent food safety risks.

Foodborne disease outbreaks affect more than 600 million of people worldwide with approximately 420,000 deaths reported every year [6–8], with developing and middle income countries probably exposed to higher risks [9–12]. In 1993, a World Health Organization (WHO) survey of 100 street foods in 100 countries, reported hygiene malpractices during storage and processing of street vended foods [6]. Foodborne illnesses are common in developing countries and several factors like poor hygiene practices at vending sites, low level of literacy, inadequate and/or poor policing of food safety laws, have all been attributed to increased foodborne diseases incidences [6, 13, 14]. Furthermore, insufficient cooking or inadequate heating or cooling of food have been reported to account for 44% of the causes of foodborne illness in developing countries [15]. Inappropriate practices involving cross-contamination, poor hygiene and reuse of food leftovers are responsible for causing 14% of these diseases. Additionally, incorrect food handling, with handlers acting as transmission vehicles of pathogens has been singled as the leading course of foodborne diseases [6, 16]. The lack of hygiene on the streets where food is prepared and sold is not only characteristic of developing countries, but also in emerging countries, such as Brazil. Studies carried out in Brazil by different authors that evaluated the microbiological quality of vendor’s hands which worked in unhygienic conditions showed fecal coliforms in 47 % and 55.6 % on hands of food handlers [17, 18].

In recent years, the consumption of ready-to eat vegetables salads has drastically increased owing to the associated health benefits and urbanization [19, 20]. However, with this increase has been the rise in foodborne disease outbreaks [21]. As an example of this, in Canada, between 2001 and 2009, there were 27 outbreaks related to the consumption of fresh vegetables and included about 1,549 cases of disease. Fresh and leafy vegetables were responsible for 26% of cases [22]. Additionally in the period of 2010 to 2017, there were 85 outbreaks related to fresh products with confirmed etiology in the United States [23].

Fresh produce that are usually consumed raw or after minimal processing can be contaminated by pathogenic microorganisms through direct and/or indirect contact with contaminated irrigation water, improperly composted animal manure and many other sources [19, 24, 25]. Lettuce is the most consumed leafy vegetable in Mozambique and in the world. Lettuce salads, add of other vegetables as tomatoes and onions, are often sold on the streets of the city of Maputo and often, during their preparation, the correct hygiene is not carried out. In Africa, studies conducted in a number of developing countries including Ghana, Burkina Faso and Nigeria, have shown that street food vendors are poorly informed on food safety [26–31]. In Mozambique, there are several food street vendors and there is paucity of food safety information due to lack of training, surveillance, documentation and reporting [32]. At the same time, daily thousands of people eat vegetable salads vended at streets and do not complain or report foodborne diseases. The objective of this study was to evaluate the knowledge, attitudes and Enterobacteriaceae contamination on the food handlers' hands and in ready-to-eat vegetables salads vended in Maputo City streets, Mozambique.

2. Materials and Methods

2.1. Sampling plan

The study was conducted in Maputo, capital city of Mozambique. A total of 110 street food vendors were selected from six different locations to participate in the survey from March 2018 to May 2018. The vending sites were chosen according to the flow of sellers and consumers. In order to assess the

safety knowledge and attitudes of these street vendors a structured questionnaire using face-to-face interviews was conducted. Participation by respondents was voluntary and took approximately 25 minutes to complete the survey per individual.

2.2. Questionnaire design

The questionnaire used to assess the food safety knowledge and attitudes in this study was adopted from Food Code [33] and other published studies [26, 34–36]. Survey consisted of 82 items, which were divided into four parts, including demographic characteristics (05 items), food safety knowledge (32 questions) and food safety attitudes (11 questions). The questionnaires were administered through by face-to-face interviews to cater for illiterate participants with difficulties in self-administering the questionnaire. The demographic section contained information about gender, age, marital status, educational level and training in food safety. The food safety knowledge section was designed to assess vendors' level of knowledge about personal hygiene, cross-contamination and sanitation, time and temperature control and foodborne pathogens. It consisted of three possible answers "yes," "no" and "do not know" to reduce the probability of respondents in selecting the correct answer by chance. The food safety attitudes section contained 11 questions and was designed to evaluate the individual vendors understanding about the hygiene and food safety. The protocol and the objectives of the study were explained to the street food vendors by data collectors, before interviews. The data collectors were trained about the objective, the importance of the study, the confidentiality of information, respondent’s right, techniques of interview, and inspection of food services. The items of the questionnaire were first written in English and then translated to Portuguese. The inclusion criterion for participation in the research was to be food handler of a selected vending area of Maputo city. Senior investigators ensured the completeness and reliability of the information gathered throughout the data collection process.

2.3. Pilot study

A pilot study was conducted with 20 food handlers from two different vending areas in order to assess validity and clarity of the questions and time requirements. Based on the answer and comments of the respondents, changes were made in questionnaire before the final version be applied.

2.4. Microbiological analysis of ready-to-eat salads and hands of food-handlers in street of Maputo City, Mozambique

Enterobacteriaceae Bacteria Plate Counts (EBPC) were carried out in ready-to-eat salads and hands of food handlers to assess the prevalence of Enterobacteriaceae and E. coli as hygiene indicators. Microbiological results were compared and correlated to the habits and attitudes of street food vendors. A total of 110 samples (55 samples of ready-to-eat vegetable salads and 55 hands samples for food handlers) from different selling points, were selected randomly. Approximately 100 g of ready-to-eat (RTE) vegetable salads samples were collected in sterile plastic bags. Typically, vegetable salad samples were constituted by lettuce, tomato, onion, vinegar, salt and oil. Ready-to-eat salads were collected because they were eaten raw, so the risk of contamination in these foods is greater. For hands samples, swabs were aseptically rubbed onto the hands of food handlers during food preparation. All the samples were immediately transported in a cooler box at 4 ºC to the laboratory of microbiology at Eduardo Mondlane University for the analysis. EBPC of the samples ready-to-eat vegetable salads and food handlers were carried out according to ISO 21528-2 Second edition (2018). The typical Enterobacteriaceae colonies showing red-purple halos were selected for counting, and purified for further identification using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). The bacterial counts were expressed as a number of colony forming units per gram (cfu/g) for RTE salads and units per square centimeter (cfu/cm2) for hand swabs.

2.6. Confirmation of presumptive EBPC using MALDI-TOF-MS

Purified bacterial cultures isolated from the EBPC were transferred in duplicate to nutrient agar media directly to the MALDI-TOF-MS steel polished

target plate (Bruker, Bremen, Germany) and overlaid with the cyano-4-hydroxycinnamic acid matrix (Bruker). The target plate was subsequently analyzed using MicroFlex LT MALDI-TOF-MS (broker) in conjunction with Biotyper automation software (Bruker). Duplicate score values were recorded and used to determine the accuracy of identification. The MALDI-TOF test was carried out in the Department of Plant and Soil Sciences, University of Pretoria.

2.7. Statistical analysis

All information regarding the demographic characteristics, food safety knowledge, practices and attitudes of vendors was collected and recorded in the Microsoft Excel worksheet (version 2010). Statistical analysis of data was performed using the Statistical Package for Social Sciences (SPSS, version 21.0 Inc. Chicago, IL, USA). Descriptive statistics (means, standard errors, percentages and frequencies) were used for summarizing the demographic characteristics, knowledge, and attitudes scores of the respondents. The mean score was calculated by summing all the partial correct answers and divided by the number of questions from the questionnaire. The mean was calculated by summing all the partial correct answers and divided by the number of questions from the questionnaire. Also, the correlation among the demographic characteristics and knowledge, attitudes and practices of food handlers was analyzed. The two-sided p-value of<0.05 was applied and considered to be statistically significant.

3. Results and Discussion

3.1. Demographic characteristics

A total of 110 street food handlers were involved in the study from six street food selling points in Maputo, Mozambique. The characteristics of the respondents are described in Table 1. Most of the respondents were female (89%) and 40% of the respondents were married. These current results are similar to other studies, that have been carried out in other parts of the world, where the female respondents exceeded 50% [31, 36, 37]. Additionally Ghatak and Chatterjee [38] and Kunadu et al. [39] reported about 50% of the street

vendors were married in China and Ghana, respectively. In our study, most of vendors were between the ages 26 to 35 (40 %), while 17% were < 26 years-old and 13% were between 36 and 40 years-old. Similar results were obtained by Smigic et al. [40], who found that about 40% of the food street vendors in three (3) European countries were <45 years-old. According to Allen and Sachs (2007) cooking meals is mainly a women's responsibility globally. Women between 25 and 40 years of age have the responsibility to support their families or supplement the family income through selling food in the streets, an activity that doesn't require training or significant investments [41].

In general, the education levels of the respondents in this study were low, which approximately 55% of the respondents having only primary school. Similar observations were reported in studies carried out in Vietnam, Brazil and Nigeria [31, 42, 43]. For example, Samapundo et al. [36] studied food safety knowledge, attitudes and practices of street food vendors in Port-au-Prince, Haiti and found that 45% of food vendors have primary school level. In addition, Cortese et al. [43] reported mostly street food vendors generally completed only elementary school in Brazil. This can be related to the fact that in many countries and mainly in developing countries, women are less educated than men because of societal, cultural setups and beliefs. Grant (2012) also reported that the low levels of formal education among urban youth in developing countries lead them to develop entrepreneurial skills that do not require large investments, such as selling food on the streets. Low level of education of most sellers could make it more difficult for vendors to understand food hygiene and safety. According to Madaki and Bavorova [44], literacy and levels of education were statistically significant determinants that increase the food safety knowledge of the food vendors in Nigeria. Regarding training, food handlers have never received any formal training. Mozambican food service legislation does not require food handlers to take any courses on good hygiene practices for food services and are allowed to operate without any formal awareness or knowledge. Food safety training for food handlers in Maputo should therefore become mandatory as knowledge is fundamental for proper practices and customer satisfaction. Several studies showed that training is an effective tool for improving knowledge [9, 45, 46]. In Southern Brazil, food handlers have to be trained for at least 16 hours on good hygiene practices in order to be

responsible for food preparation and handling in the food services sector [47]. In order to be effective, training should not only focus on theoretical aspects of knowledge but should also be practical to promote positive attitudes and good food safety practices that can assure customer satisfaction and consistent income [4]. According to our results, the vendors had a mean food safety knowledge score of 42.58 ± 20.83, which indicates a general lack of food safety knowledge as well as a general lack of related knowledge similar to other studies as was reported in Nigeria with restaurant staff [48]. The general lack of knowledge is due to the fact that food handlers have not received any training in good hygiene practices and food as well as potential pathogens associated with food production, processing and consumption. Several studies have suggested that training of employees are effective tools to improve food safety [45, 49–51]. However, in many parts of the world, principally in developing countries such as Mozambique, food handlers are not trained on food safety [50, 52, 53]. Moreover, limited food safety knowledge primarily increases the risk of foodborne illnesses [54]. In this context, there is a need for mandatory training considering the increased number of sellers in streets in Maputo City.

Table 1
Demographic characteristics of 110 food handlers in street of Maputo city, Mozambique.

Demographic Characteristics	Frequency	Category	Percentage (%)
Gender	11	Male	11
	98	Female	89
Age (years)	19	15-25	17
	44	26-35	40
	15	36-45	14
	12	46-55	11
	20	56 - 60	18
	0	> 60	0
Educational level	60	Primary school	55
	22	Secondary	20
	19	University	17
	9	Illiterate	8
Marital status	44	Married (a)	40
	32	Single	29
	11	Union fact	10
	20	Divorced	18
	3	Vidower (a)	30

3.2 Food safety knowledge of food handlers in Maputo City, Mozambique

In this study, the total average food safety knowledge of food handlers was found to be unsatisfactory with a mean score of 42.58 ± 20.83 (Table 2). A total of 62.86% of respondents were aware, while 12% were unaware of the presence of foodborne pathogens in foods (Fig. 2).

3.2.1 Personal hygiene of food handlers

Hands play an important role in the transmission of foodborne pathogens. Therefore, any activity which may contaminate the hands must be followed by thorough hand washing [33]. Our findings show that most of the participants (78.2 %) knew that it was necessary to wash hands before preparing food to reduce the risk of food contamination (Table 2). More than half of participants (62.7 %) knew that it was important to wash hands after using the toilet and after handling raw meat. As is known, some pathogens are frequently transmitted by food contaminated by infected people [33]. Food handlers are potential vehicles for transferring pathogenic microorganisms and intestinal parasites to food [33, 55]. Additionally, in our study, food handlers had shown acceptable knowledge about the importance of washing hands after handling the garbage (68.2 %), wearing gloves before touching ready-to-eat foods (70.9 %) and use of different knifes to cut raw meat, poultry and vegetables (56.4 %). However, less than half (40.9 %) of respondents knew that it was important to wash hands after touching any part of the body. Similar studies conducted by Andoy and Valmorida [56] evaluated the food safety knowledge of street food vendors in Ozamis City, southern Philippines, and concluded that street food vendors are knowledgeable about the basic standards on health and personal hygiene. Similar results were found in studies where food handlers gave satisfactory answers about good personal hygiene in Kuwait and Indonesia [45, 57]. The data obtained from the current study indicates that although the total average food safety knowledge of vendors was unsatisfactory, they presented a relatively high percentage (62.86%) of knowledge of personal hygiene. However, there is need to improve on the number of knowledgeable sellers through continuous training of vendors.

3.2.2 Cross-contamination and sanitization

The overall knowledge about cross-contamination and sanitization was low (Fig. 2). About 45 % of respondents were able to answer all of the cross-contamination and sanitation questions correctly (Table 2). It was observed that most of them knew that it is important to wear protective clothing to reduce the risk of food contamination (69.1 %) and that it is not correct coming to work if they have fever, coughing or a cold (67.3 %). Although many participants demonstrated a high level of knowledge about cross-contamination, general observations in the site visits showed that most of the vendors had dirty clothes, that can be a source of contamination of specific food batches. A few vendors knew that it was important to wash knifes used to cut raw meat or poultry with water and soap before using it to cut vegetables (32.7 %), and that it is essential to wash food contact surface with water and soap followed by application of sanitizer (27.3 %). Moreover, 42.7 % of respondents knew that raw foods must be stored separately from ready-to-eat foods in the refrigerator. Based on our results, it was clear that there is a lack of knowledge about cross-contamination, and this knowledge is important to prevent food outbreaks. Cross-contamination is a major concern in food production areas [58] and can be a significant risk factor during the postharvest processes leading to foodborne outbreaks [59]. According to Carrasco et al. [60], cross-contamination episodes have been connected with poor sanitation practices, equipment design and control of ingredients. Due to improper handling, ready-to-eat foods are susceptible to contamination by pathogenic microorganisms, making them unfit for consumption. Therefore, it is important preventing cross-contamination and recontamination of food, increasing levels of food safety.

3.2.3 Time and temperature control

Food temperature is one of the most important factors to control and prevent foodborne illness in food services [61]. In this study, only 35.68 % of respondents rightly answered the importance of temperature control of foods to reduce food spoilage, especially in salads (Fig. 2). Most respondents did not know that: it is incorrect to leave raw meat or poultry on the kitchen counter (70.2 %), it is important to keep the refrigerator operating temperature between

1 and 5 °C (57.3 %) and it is important to maintain the freezer operating temperature -18 °C or less (55.5 %) (Table 1). Although few respondents knew about the importance of time and temperature control, most respondents (63.3 %) acknowledged the importance of preserving leftover food in the refrigerator. Similar findings were reported by Al-Kandari et al. [45]. In their study, the majority of the respondents were not aware of the importance of the time and temperature control required for preventing the microbial growth in foods.

During our research, it was noted that street vendors did not have the proper equipment to keep food sold under appropriate temperature conditions, as recommended in general food regulations, such as Ordinance 78/2009. Most foods, such as rice, roast beef, chicken and lettuce salad, sold to consumers in these locations, was kept at room temperature. It was also observed that, in many cases, street vendors kept food inside the cars they used to transport. This practice is risky and can compromise the quality and safety of food, especially for a country with an average annual temperature of 28 ºC, as in Mozambique. According to the FDA [30], food kept without temperature control allow temperature fluctuations during the day and may cause an increase in microbial density representing a bigger risk to consumers. Based on this, the Food Code (2017) in USA and the ordinance 78/2009 in Brazil (Portaria 78/2009) preconized that ready-to-eat foods must be kept and distributed at refrigerated temperatures < 5 ºC or above 60 ºC. Therefore, the use of adequate temperatures in food services is important and reduces the risk of bacterial multiplication, thus ensuring that consumers consume safe food [22].

3.2.4 Knowledge of foodborne pathogens

In this study, only 30.80% of respondents answered the questions about the knowledge of foodborne pathogens (Fig. 2). Regarding knowledge of foodborne pathogens, the majority of vendors did not know about Shigella, Salmonella and Staphylococcus being food pathogens (87.3%, 92.7%, 88.2%, respectively) (Table 2). Similar results were reported by Asiegbu et al. [62], who found that over 70% of the street food vendors in the Johannesburg municipality have never heard about Salmonella spp., Escherichia coli, Listeria monocytogenes and Campylobacter jejuni. Only 32.7% of the respondents knew that abortion in pregnant women can be induced by foodborne diseases

and only 10.9% knew that typhoid fever can be transmitted by Salmonella present in food. On other hand, most (55.5%) of them knew that AIDS is not a foodborne disease and children, pregnant women, older individuals and healthy adults are not at equal risk for getting a foodborne disease (53.6%). The knowledge level of respondents in current study is comparable to results reported previously in Ho Chi Minh city, Vietnam by Samapundo at al. [63] and Osaili et al. [34], who found that all vendors did not know or did not believe that hepatitis virus type A (97.5 %), Salmonella spp. (97.5 %) and Staphylococcus spp. (100 %) are foodborne pathogens. Actually, issues related to food pathogens are not easy to be understanding by food handlers that have never be trained. The use of correlations between microbes and knowledge and finally appropriate attitudes can be a good method to be adopted in trainings in order to initiate a good capacitation of food handlers.

Table 2
Food safety knowledge of 110 food street vendors in Maputo City, Mozambique

Category	Questions	Responses n (%) Yes	No	Do not know
Personal hygiene	1 - Is it necessary to wash hands before the start of preparing foods to reduce the risk of food contamination?	86 (78.2)	14 (12.7)	10 (9.1)
	2 - Is it important to wash your hands after using the toilet?	69 (62.7)	21 (19.1)	20 (18.2)
	3 - Is it important to wash hands after handling raw meat?	69 (62.7)	23 (20.9)	18 (16.4)
	4 - Is it important to wash hands after touching any part of the body?	45 (40.9)	55 (50.0)	10 (9.1)
	5 - Is it important to wash hands after handling the garbage?	75 (68.2)	15 (13.6)	20 (18.2)
	6 - Is it important to wear clean gloves before touching ready to eat foods?	78 (70.9)	22 (20.0)	10 (9.1)
Cross contamination And sanitation	7 - Is it important to use a different knife to cut raw meat or poultry and vegetables?	62 (56.4)	38 (34.5)	10 (9.1)
	8 - Is it important to wash the knife used to cut raw meat or poultry with water and soap before using it to cut vegetables?	3 6 (32.7)	6 6 (60.0)	8 (7.3)
	9 - Is it important to use different bowls to put or cut raw meat or poultry and vegetables?	50 (45.5)	44 (40.0)	16 (14.5)
	10 - Is it important to wash the knife used to cut raw meat or poultry with water and soap before using it to cut vegetables?	36 (32.7)	66 (60.0)	8 (7.3)
	11 - Is it important to wash food contact surface with water and soap and followed by the application of a sanitizer?	30 (27.3)	75 (68.2)	5 (4.5)
	12 - Is it important to store leftover foods and raw foods separately in the refrigerator?	47 (42.7)	50 (45.5)	13 (11.8)
	13 - Is it important to wearing protective clothes (cap, mask, gloves) when handling food to reduce the risk of food contamination?	76 (69.1)	25 (22.7)	9 (8.2)
	14 - Is it correct coming to work during the infectious sick (fever, coughing, cold, diarrhea, etc.?	74 (67.3)	13 (11.8)	23 (20.9)
Time and temperature control	15 - It is correct to leave raw meat or poultry at the kitchen counter?	78 (70.2)	20 (18.2)	12 (10.9)
	16 - Is it correct to thaw frozen raw meat or poultry in the refrigerator?	55 (50.0)	34 (30.9)	21 (19.1)
	17 - Is it important to keep the refrigerator operating temperature between 1 and 5°C?	45 (40.9)	2 (1.8)	63 (57.3)
	18 - Does pre-cooked food reduce the risk of food contamination?	64 (58.2)	25 (22.7)	21 (19.1)
	19 - Is it important to keep the freezer operating temperature −18 °C?	37 (33.6)	12 (10.9)	61 (55.5)
	20 - Is it important to keep leftovers in the fridge?	70 (63.6)	24 (21.8)	16 (14.5)
	21 - Is it safe to store leftover food on the stove in the kitchen?	44 (40.0)	46 (41.8)	20 (18.2)
	22 - Can reheating cooked food contribute to food contamination?	64 (58.2)	25 (22.7)	21 (19.1)
Foodborne pathogens	23 - Can abortion in pregnant women be induced by foodborne diseases?	36 (32.7)	66 (60.0)	8 (7.3)
	24 - Can bloody diarrhea be caused by food?	53 (48.2)	39 (35.5)	18 (16.4)
	25 - Is *Shigella* related to foodborne diseases?	14 (12.7)	28 (25.5)	68 (61.8)
	26 - Is Hepatitis A virus related to foodborne diseases?	35 (31.8)	52 (47.3)	23 (20.9)
	27 - Is *Salmonella* among food pathogens?	8 (7.3)	59 (53.6)	43 (39.1)
	28 - Is *Staphylococcus aureus* among the food pathogens?	13 (11.8)	24 (21.8)	73 (66.4)
	29 - Can typhoid fever be transmitted by food?	12 (10.9)	24 (21.8)	74 (67.3)
	30 - Is AIDS a foodborne disease?	33 (30.0)	61 (55.5)	16 (14.5)
	31 - Are children, healthy adults, pregnant women and older individuals at equal risk for getting a foodborne disease?	59 (53.6)	43 (39.1)	8 (7.3)
				42.58 ± 20.83 (Average ± Standard Deviation)

3.3. Food safety attitudes of food handlers in Maputo city street, Mozambique

In this research we assumed that attitudes of food handlers were influenced by their beliefs and they were investigated. The street vendors had a food safety attitude mean score of 67.8 %, which indicated that most food handlers have positive attitudes that contribute to food safety. The results are shown in Table 3. For example, approximately 53.6 % of street vendors agreed that raw eggs should be stored separately from other foods to reduce the risk of food contamination. A higher proportion of vendors believe that proper hand hygiene can prevent foodborne diseases (89.1 %) and food handlers with abrasions or cuts on hands should not handle foods (78.2 %). In addition, most of the respondents agreed that protecting hair is an important practice to reduce the risk of food contamination (78.2 %) and knives and cutting boards should be properly sanitized to prevent cross-contamination (66.4%). Finally, 85.5 % replied that they will be ready to correct any wrong food handling practices. Similar results, which most food handlers showed positive attitudes, were obtained by Osaili et al. [26, 34] and Abdul-Mutalib et al. [64] in Brazil and Malaysia, respectively. However, attitudes towards food safety were generally negative in a study conducted in Ghana where the researchers evaluated the food safety knowledge, attitude and practices of food handlers from institutional food service establishments, serving hospitals, boarding senior high schools and prisons [39]. In summary, good attitudes can contribute significantly to the adherence to hygienic-sanitary conditions [61], as food handlers with higher specific attitudes have better knowledge about food safety.

Table 3

Assessment of 110 food safety surveys concerning the attitudes of street vended food handlers in Maputo City, Mozambique

	Number of responses (%)		
Questions	**Yes**	**No**	**Do not know**
I believe, proper hand hygiene can prevent food borne diseases	98 (89.1)	5 (4.5)	7 (6.4)
I believe raw and cooked foods should be stored separately to reduce the risk of food contamination.	59 (53.6)	24 (21.8)	27 (24.5)
I believe participating in training program of food safety will benefit my food safety and will increase my knowledge prevent of food borne disease	82 (74.5)	16 (14.5)	12 (10.9)
I agree workers health status must be checked at regular intervals.	74 (67.3)	13 (11.8)	23 (20.9)
I believe towels used for the cleaning of dishes can be a source of food contamination.	83 (75.5)	9 (8.2)	18 (16.4)
I believe personal protective equipment and clothes reduces the risk of food contamination.	78 (70.9)	22 (20.0)	10 (9.1)
I agree food handlers with abrasions or cuts on hands should not handle foods.	86 (78.2)	17 (15.5)	7 (6.4)
I believe cooked foods properly reduce the risk of food borne diseases.	78 (70.9)	22 (20.0)	10 (9.1)
I believe keep cooked food above 60°C is safe for the consumer.	79 (71.8)	12 (10.9)	19 (17.3)
I believe washing eggs as soon as possible after purchase is a safe practice	71 (64.5)	26 (23.6)	13 (11.8)
I believe sanitized properly knives and cutting boards is important to reduce risk of food contamination.	73 (66.4)	19 (17.3)	18 (16.4)
I believe wearing gloves when handling ready-to-eat foods is an important practice to reduce the risk of food contamination.	69 (62.7)	21 (19.1)	20 (18.2)
3 I will be ready to correct any wrong food handling practices that I have been doing after learning about food safety.	94 (85.5)	7 (6.4)	9 (8.2)
Mean score of food safety attitudes	67.83 ± 17.80 (Average ± Standard Deviation)		

3.4 Microbiological assessment of street food salads and food handlers in Maputo City

The prevalence of *Enterobacteriaceae* observed in salads ready for consumption and on the hands was 92.7% and 61.8%, with average counts of 4.23 log10 CFU / g and 3.14 log10 CFU / cm^2 respectively (Table 5). Similar results were obtained by other authors, such as Shiningeni et al. (SHININGENI et al., 2019b), Ssemanda et al. (SSEMANDA et al., 2018) and Santos et al. (SANTOS et al., 2012), when investigating the prevalence of pathogenic bacteria in ready-to-eat salads on the streets in different African countries. They found the mean *Enterobacteriaceae* counts of 4.10 - 4.23 log10 CFU/g, 3.3 - 4.23 log10 CFU/g and 5.44 4.23 log10 CFU/g, respectively. According to International Commission of Microbiological Specification for Foods, salads are considered unsatisfactory when levels of *Enterobacteriaceae* are ≥ 4 log_{10}

CFU/g (ICMSF, 2006). In our study we detected counts above this limit in 40% of (22/50) RTE lettuce salads samples tested. The predominant *Enterobacteriaceae* isolated in ready-to-eat salads and hands were *Klebsiella pneumoniae* (41 % and 29 %, respectively) and *Enterobacter cloacae* (27 % and 38 %, respectively). Similar results were reported by Al-Kharousi et al. (2016) that found *Enterobacteriaceae* (*Enterobacter cloacae, E. coli* and *Klebsiella pneumoniae)* in the majority (91 %) of 105 samples of imported fresh fruits and vegetables originated from different countries in the world including local samples in Oman. Zekar et al. (2017) evaluated the contamination levels of fruits, vegetables in 491 samples collected from 5 markets in Bejaia area, north-eastern Mediterranean coast of Algeria and found that *E. coli*, *Enterobacter cloacae*, *Enterobacter asburiae* and *Klebsiella pneumoniae* were reported as the dominant species in fresh vegetable. Garayoa et al. (2017) assessed the routine food safety procedures carried out in catering services in Spain and founded high levels (> 4 CFU/cm^2) of *Enterobacteriaceae* on hand-contact surfaces such as aprons and handles drawers.

Although many genera of *Enterobacteriaceae* family cannot cause foodborne diseases, they can be indicators of raw material poor quality and handling practices, including hygiene and/or temperature and time control (ANAND; GRIFFITHS, 2011). Moreover, some *Enterobacteriaceae,* as *Salmonella* and *Shigella,* are important food pathogens, while other, as *E. coli,* are known as opportunistic pathogens which may raise concern to improve the microbial quality of fresh produce and have been associated with foodborne disease outbreaks linked to fresh produce (TOPE; HITTER; PATEL, 2016). Additionally, in ready-to-eat salads the *Enterobacteriaceae* can be transmitted by the food handler's hands with poor hygiene habits, by flying insects or rodents, or even by water (EASA, 2010). Although, Mozambican legislation does not set out standards for this family of microorganisms, the study of these bacteria is relevant, since they can indicate poor hygiene practices during or after food production (CAMARGO et al., 2014).

In our study, *E. coli* was detected in only one sample of the food handler hands (Table 6), and this result was surprising because the presence and mainly the high counts of generic *E. coli* are frequently related to inadequate good hygienic practices, as some of the situations observed in our study. This

result may be partially explained because the great majority of food handlers declared to be aware and to wash their hands before, during and after touch contaminated objects and foods. Even though the *E. coli* contamination was low, attitudes and procedures about food safety presented inadequacies, indicating there is a need for further measures for the development of hygienic-sanitary control during the preparation of foods in streets of Maputo. In addition, multidimensional programs to improve the conditions for the sale of food on the streets of Maputo city should be designed to improve the conditions of food sale, and food handlers must be more careful to preserve the health of their consumers.

Table 5

Prevalence and counts of *Enterobacteriaceae* in ready-to-eat vegetable salads and hands samples from food handlers in Street of Maputo City, Mozambique

Sample type	Number of samples	*Enterobacteriaceae* prevalence (%)	% unsatisfactory (≥ 4 log cfu/g)	Mean Count (log cfu/g)	Min-Max
Ready-to-eat salad	55	92,73	40,00	4,23 ± 0,78	2,00 ± 6,15
Hands	55	61,81	-	3,14 ± 0,74	1,60 ± 4,48

Table 6

Distribution of bacterial strains identified in samples of ready-to-eat (RTE) vegetable salads and hands of food handlers in street of Maputo City, Mozambique

Identified strains/sample	RTE Salad	RTE (%)	Hands	Hands(%)	Total	Total (%)
Citrobacter freundii	3	5,88	3	8,82		7,06
Enterobacter asburiae	5	9,80	4	11,76		10,59
Enterobacter cloacae	14	27,45	13	38,24	27	31,76
Enterobacter ludwigii	2	3,92	0	0,00		2,35
Escherichia coli	0	0,00	1	2,94		1,18
Klebsiella oxytoca	4	7,84	1	2,94		5,88
Klebsiella pneumoniae	21	41,18	10	29,41	31	36,47
Kosakonia cowanii	0	0,00	2	5,88		2,35
Raoultella ornithinolytica	1	1,96	0	0,00		1,18
Raoultella terrigena	1	1,96	0	0,00		1,18
Total	51	100,00	34	100,00	85	100,00

3.7. Correlation among knowledge, attitudes and microbiological quality of ready-to-eat vegetable salads and hands of food handlers in Street of Maputo City, Mozambique

No significant positive correlation were found between knowledge, attitudes (rs = 0.011, P > 0.05) and microbial loads on ready-to-eat salads and food handlers hands (rs = 0.219, p > 0.05) in the present study (Table 7). Other studies reflected a positive correlation between knowledge, attitudes and bacterial contamination [74, 75]. Our results are in agreement with [76] who investigated the relationship between knowledge, attitudes and practices of food handlers in developing countries.Approximately two hundred and fifty-three articles on knowledge, practices and attitudes of street food vendors in developing countries were reviewed. The authors concluded that fifty percent of the articles examined did not translate knowledge into attitudes or practices, even after some training in food security. This contradiction may be related to the lack of basic facilities, such as water and toilets at food outlets in these countries.

Table 7

Correlation among knowledge, attitudes and microbiological quality of samples of ready-to-eat vegetable salads and hands of food handlers in street of Maputo city, Mozambique

Level	Spearman's rho	Sig.*
Knowledge – Attitudes	0.011	0.000
Salads – Hands	0.219	0.000

*correlation is significant at the 0.01 level (2 – tailed)

4. Conclusions and recommendations

This study carried out an assessment of the food safety knowledge and attitudes and the microbiological quality of RTE vegetable salads and hands of food handlers of street food vendors in Maputo. Assessment of street food vendors revealed that the level of knowledge and attitudes of food handlers in Maputo streets was unsatisfactory for several food safety aspects. Limited knowledge was noted regarding foodborne pathogens, time and temperature control as well as cross-contamination. On the other hand, satisfactory knowledge about personal hygienic procedures were observed from the majority of respondents. Although most samples were positive for the presence of *Enterobacteriaceae*, unsatisfactory levels were detected in less than half of the lettuce salad samples tested, and *E. coli* was only detected in one sample collected from a hand of a food handler. These results indicate that other factors are affecting the prevalence of this important indicator organism more commonly tested for in the food services sector. However, further studies are required to understand why no correlation existed between poor knowledge and attitudes of food safety and *E. coli* levels obtained during this study. This research is the first in Maputo that presents a baseline data for further investigations on food handler's knowledge and attitudes and the microbiological assessment of street vendors and food and could help the government of Mozambique to identify factors influencing food hygiene practices and the development of guidelines for the production and sale of street vended foods. This study also demonstrated the need for training street vendors in good hygiene practices and food safety that can be able to sustain the quality and safety of Mozambique street foods.

5. Conflicts of interest

The authors declare no conflict of interest.

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8. Ethical issues

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4.3 Artigo 3

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Prevalence of *Enterobacteriaceae* on Ready to Eat Salads, Drinking Water and Surfaces in Food Markets of Maputo, Mozambique

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Abstract Vegetable salads constitute an important component of many meals worldwide. However there is concern for their safety and microbiological quality because they have been implicated in outbreaks of many foodborne diseases, especially in developing countries. In Mozambique, the knowledge of the microbiological quality and virulence genes of bacterial isolates from ready-to-eat (RTE) salads is limited. This study aimed to evaluate the prevalence of *Enterobacteriaceae* on RTE lettuce, drinking water and surfaces in food markets of Maputo, Mozambique. A total of 35 samples of RTE lettuce salads and 42 drinking water samples were collected from 35 food vendors, in addition to 105 swabs of hands, knives and bowls from seven markets in Maputo City, Mozambique. The prevalence of *Enterobacteriaceae* bacterial isolates from the collected samples was determined using plate counts method following ISO 21528-2 and ISO 21528-1 (for drinking water). The purified isolates were identified using a matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF-MS). A total of 219 isolates were obtained. *Enterobacter* isolates (45.2%) were the predominant species. *Enterobacteriaceae* counts ranged from 0.52 to 6.98log CFU/g. There was no statistically significant correlation between bacteriological counts on RTE lettuce salads and swabs. However, there were significant differences among the numbers of *Enterobacteriaceae* detected in water for other samples. The prevalence of *Escherichia coli* was observed in fewer samples, a remarkable tendency of the presence of this bacterium was found in the utensils. The *E. coli* isolates obtained in this study tested negative for the presence of virulence genes (stx1F, stx1R, stx2F, stx2R). These findings provide valuable background information that can support food safety decisions and confirm that the vast majority of vendors do not sanitize utensils effectively.

Keywords: *food markets, indicators, MALDI-TOF-MS, hygiene quality, foodborne pathogens*

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1. Introduction

The consumption of ready-to-eat has increased in developing countries due to changing lifestyle patterns [1]. Vegetables salads are regarded as an essential part of a nutritious and healthy worldwide [2,3,1]. Most salads are however consumed raw or after minimal processing, and generally do not receive heat treatment before consumption [4,5]. Salads derived from lettuce have been linked to numerous foodborne disease outbreaks associated with *E. coli* O157:H7 [6,7,8]. In 2008, the United Nations ranked green leaves as the “highest priority” for the number of outbreaks and the types of microbial hazards [9,7]. The Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) have investigated several multistate outbreaks involving vegetables, salad mix, in the past three years (2016-2018) and found Norovirus, *Salmonella* and *Escherichia* as the main cause of the foodborne diseases [7].

Contamination of RTE vegetable salads can occur through various production routes. Contamination may originate from human, animal, and environmental sources [1]. Food preparation facilities in food service are also responsible for contamination of salads which may affect the quality and lead to food safety issues [10,11]. Inaccessibility to safe water, lack of agricultural infrastructure largely contributes to contamination of vegetables salads in the developing countries [12].

Salads sold can be unfit for human consumption and could be deleterious to the health of consumers. Abakari et al., conducted a study in Ghana and found *Escherichia coli* in 96.7% of salad samples with levels ranging from 0 to 7.56 $\log_{10}$ CFU/g. *Salmonella* spp. and *Shigella* spp. were present in 73.3% and 76.7% of salads, respectively [13].

Members of the *Enterobacteriaceae* family are a gram-negative, non-spore forming bacterium that includes many bacteria that are found in human or animal intestinal tracts, as well as plants and the environment [14].

The *Enterobacteriaceae* may be superior to coliforms as indicators of sanitation indicated by good manufacturing practices because they have collectively greater resistance to the environment than the coliforms. However, coliforms constitute an important group within the *Enterobacteriaceae* family and constitute about 10% of the intestinal microbiota [15,14]. Important food pathogens in the *Enterobacteriaceae* family include *Cronobacter* spp, *Escherichia coli, Salmonella enterica*, *Shigella (boydii, flexneri, sonnei and disenteriae)* and *Yersinia* (*enterocolitica* and *pseudotu-berculosis*) [16,17,18]. In RTE salads, *Enterobacteriaceae* pathogens, including *Escherichia*, *Salmonella* and have been implicated in disease outbreaks [19].

Species that are part of the coliform group include *Citrobacter, Enterobacter, Hafnia, Klebsiella* and *Escherichia*. These bacteria are used as indicators of food health quality because they are abundant in the feaces of warm-blooded animals and they are relatively quick and simple to detect [16]. The presence of coliforms in food points to failure to comply with proper good hygienic practices. Indicators are used for a variety of purposes in food systems including evaluating quality or safety of raw or processed food products and validating effectiveness of microbial control measures [18]. Other members of this family can be found in aquatic environments, soil, and vegetation [17].

Foodborne diseases are a major public health concern and costs billions of dollars losses every year [20]. The identification of bacterial pathogens from food has been traditionally done using culturing of the microorganisms on a selective media. However, traditional methods are time consuming, costly and not sensitive [20,21,22]. With the increased outbreaks of foodborne diseases, fast, reliable and accurate monitoring and detection of foodborne pathogens in food cannot be overemphasized. Recently, several rapid detections, identification, and monitoring methods like Immunoassays methods, DNA-based detection methods, MALDI-TOF MS biosensors methods; Electrochemical biosensors have been developed for f foodborne pathogens [23,24,25,26,27,28].

Matrix-Assisted Laser Desorption Flight Time Mass Spectrometry (MALDI-TOF MS) has become one of the widely used and preferred methods for identification of food borne pathogens, since it allows rapid and accurate identification of microorganisms to the species level in clinical microbiology laboratories [29,30,31,32,33,34]. It has been successfully used in clinical diagnosis, food safety control, environmental monitoring [35,36,37,38,39]. Studies on microbiological quality and virulence genes in bacterial isolates of ready-to-eat salads provided by vendors in markets of Maputo, Mozambique are limited. However, Macaza found high counts of *Enterobacteriaceae* of *E. coli* in samples collected from food markets in the Nampula city [40], which indicates that the conditions are unsatisfactory. Food markets in Mozambique are described as establishments where people, in general, will have breakfast and lunch. Therefore, this study aims to determine the microbiological quality (based on hygiene indicator bacteria) and the prevalence of potential human pathogenic bacteria in RTE lettuce salads, drinking water and surfaces in food markets at Maputo, Mozambique. It is envisaged that the information from this research will be useful in providing recommendation on effective mitigation efforts toward enhanced food quality in vended food in Mozambique.

2. Materials and Methods

2.1 Sampling design

Seven markets were visited and in each market twenty seven (n = 26) samples were collected in Maputo, Mozambique, over a 6-month period from March to August 2019. To obtain a representative sample for a given market, we randomly purchased the samples in different points of the markets. A total of 182 samples were collected in this study period (Table 1). Generally, in Maputo, Mozambique, food markets are open-air, made up of small establishments or small food service called “stalls.” A sample from each vendor consisted of a RTE salad (lettuces salads), water used in the salad making process, and swabs from knives used for cutting vegetables, hands, and bowls used for mixing the salad ingredients. The salads considered in this study composed of lettuce, onions and tomatoes mixtures. Samples were collected from 5 vendors in each market. Samples were collected in sterile bags kept in ice chest, maintained at 0–4 °C and taken to the Laboratory of Microbiology and Safety of the University Eduardo Modlane, Maputo campus, Mozambique and processed within 2–4 h for microbial analysis. Swab samples were done using SpongeSicle swabs with 10 ml neutralizing buffer. A verbal consent was obtained from officials responsible for the markets.

Table 1

Numbers of samples collected from seven food markets in Maputo

Source of sample/Total No of sample						
Location/Market	Salad	Water fountain	Water reservoir	Swab hand	Swab knife	Swab bowl
Museu	5	1	5	5	5	5
Povo	5	1	5	5	5	5
Mandela	5	1	5	5	5	5
Estrela vermelha	5	1	5	5	5	5
Benfica	5	1	5	5	5	5
Xipamanine	5	1	5	5	5	5
Peixe	5	1	5	5	5	5
Total	35	7	35	35	35	35

2.2 Microbiological analysis

2.2.1 Enumeration of *Enterobacteriaceae* in drinking water, ready-to-eat salads and swabs

Enumeration of *Enterobacteriaceae* colony in RET lettuce salads and swab samples was done using validated ISO methods. ISO 21528-2 Second edition (2018) was used for analysis of read to eat salads and swabs while ISO 21528-1 was used for analysis of drinking water. Briefly, violet red bile glucose (VRBG – Oxoid LTD, England) agar was prepared following the protocol recommended by the manufacturer. For RTE lettuce salads, a 25 g sample was aseptically cut from the lettuce salad using a sterile scapel, and 225 ml of buffered peptone water (3M, St.Paul, MN) was added to a sterile polyethylene bag and macerated using a stomacher 400 circulator (Seward, London, UK) at 135 rpm for 3 min. Following the standard dilution method, 1ml from each of the macerated lettuce salad were added to buffered peptone, and total *Enterobacteriaceae* count was determined by plating in to VRBG agar plates in duplicates. Swab samples and drinking water were plated directly. Plates were incubated at 37°C for 18-24 h and enumerated following the ISO 21528-2 Second edition (2018). For each of the samples analysed two colonies showing red-purple halos (presumptive indication of the presence of *Enterobacteriaceae*) were selected, purified and preserved in glycerol at -20^{o} C. The isolate identities were determined using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS).

2.2.2 Confirmation of presumptive *Enterobacteriaceae* colonies using MALDI-TOF-MS

Purified bacterial cultures stored on Nutrient Agar media (NA-Mindrad) were transferred directly to the matrix-assisted laser desorption-ionization time of flight (MALDI-TOF) steel polished target plate (Bruker, Bremen, Germany) and overlaid with the cyano-4-hydroxycinnamic acid matrix (Bruker). The target plate was subsequently analyzed using MicroFlex LT MALDI-TOF-MS (broker) in conjunction with Biotyper automation software and libray (Bruker). Duplicate score values were recorded and used to determine the accuracy of identification. A score value between 1.999 and 1.700, and value above 2.0 was used to determine the genus and probable species of the organism. Scores above 2.3 were used for highly probable species identification. The MALDI-TOF-MS test were carried at the Centre of Excellence in Food Security, Department of Plant and Soil Sciences, University of Pretoria.

2.2.3 Molecular identification of virulence genes in *E. coli* isolates

Virulence genes in *E. coli* (mdfR, mdhR, stx1F, stx1R, stx2F, stx2R) were detected using PCR. Total genomic DNA was from pure cultures was extracted using Zymo Kit – Quick DNA Minipre Kit following the manufacturer's protocol recommendation. The virulence genes were amplified using specific primers Amplification reactions were performed in a total volume 25ul of PCR green master volume of primers used. Amplification reactions were carried out in (c1000 Touch) thermocycler of the Centre of Excellence in Food Security, Department of Plant and Soil Sciences - Pretoria University. Amplification of the expected band size signifies the presence of virulence genes.

2.4 Statistical analyses

Counts of colony forming units were done in duplicate and average means and standard deviation (±SD) for each of the sample and locations were calculated. Data analysis was performed using the Statistical Package for Social Sciences (SPSS, Inc. Chicago, IL,USA). Descriptive statistics (means, standard errors, percentages and frequencies) were calculated for all variables.

3. Results

3.1. Sample collection

A total of 182 samples (n = 35 RET lettuce salads; n = 42 drinking water; n = 35 swab hands; n = 35 swabs bowls and N= 35 knifes swabs) were collected and 222 presumptive *Enterobacteriaceae* isolates of microorganisms were obtained and identified. None of the RTE salads were stored at refrigeration temperature at the point of sale. The isolates were identified up to the species level and included: *Enterobacter cloacae* (32.43%), *Klebsiella pneumonia* (20.27%), *Enterobacter asburiae* (12.16%), *Citrobacter freundii* (6.3%), *Klebsiella oxytoca* (4.95%), *Kluyvera ascobarta* (4.05%), *Escherichia coli* (3.15%). Other isolates like *Kluyvera, Kosakonia, Citrobacter, Pantoea*, *Aeromonas, Leclercia*, *Acinetobacter, Raoultella*, *Kosakonia*, *Pseudomonas,* and *Streptomyces* were isolated in low frequencies (Table 2).

Table 2
Distribution of bacterial strains identified in sampled by locations

No. of identified strains/Locations	***Museu***	***Povo***	***Mandela***	***E.V***	***Benfica***	***Xipamanine***	***Peixe***	***Total individual strains***	***% of individual strains***
Acinetobacter junii	1	0	0	1	0	0	0	2	0.9
Acinetobacter pittii	2	0	0	0	0	0	0	2	0.9
Aeromonas caviae	2	2	0	1	0	0	0	5	2.25
Citrobacter freundii	1	2	1	4	1	1	4	15	6.3
Citrobacter koseri	0	0	0	0	0	1	0	1	0.45
Enterobacter asburiae	2	4	2	1	7	4	7	27	12.16
Enterobacter cloacae	10	14	4	17	10	11	6	72	32.43
Escherichia coli	2	2	0	1	0	0	2	7	3.15
Klebsiella oxytoca	0	5	2	2	1	0	1	11	4.95
Klebsiella pneumoniae	7	16	4	10	2	5	1	45	20.27
Kluyvera ascorbata	0	1	0	1	6	1	0	9	4.05
Kluyvera cryocrescens	0	0	1	0	0	0	0	1	0.45
Kluyvera georgiana	1	0	0	0	0	0	0	1	0.45
Kluyvera intermedia	0	0	2	0	0	0	0	2	0.9
Kosakonia cowanii	0	0	1	1	0	0	0	2	0.9
Leclercia adecarboxylata	0	0	0	3	0	1	0	4	1.80
Pantoea ananatis	1	1	0	0	1	0	0	3	1.35
Pantoea cálida	0	1	0	0	0	1	0	2	0.9
Pantoea dispersa	0	0	0	0	0	2	0	2	0.9
Pantoea gaviniae	2	0	0	0	0	0	0	2	0.9
Pantoea séptica	0	0	1	0	0	2	0	3	1.35
Pseudomonas mendocina	0	0	0	0	0	1	0	1	0.45
Raoultella ornithinolytica	1	1	0	0	1	0	0	3	1.35
Streptomyces violaceoruber	0	0	0	1	0	0	0	1	0.45
Total	**32**	**49**	**18**	**43**	**29**	**30**	**21**	**222**	**100**

3.2 Total plate count and identification of *Enterobacteriaceae* on RTE lettuce salads

A total of thirty-five (n = 35) sample of RTE lettuce salads was analyzed in this study. The results show that, most (91.42%, n= 32) of the samples were found to be positive for *Enterobacteriaceae.* The identified species include: *Enterobacter cloacae* (34%)*, Klebsiella pneumonia* (28%), *Enterobacter asburiae* (18%), *Citrobacter freundii* and *Klebsiella oxytoca* (6%). Other species like *Citrobacter koseri, Kosakonia cowanii*, *Raoultella ornithinolytica* and *Raoultella terrigena* were found in 2%. The Povo and E.V markets, were the markets that had the largest number of species identified (between 1 and 4), the remaining obtained between 1 and 2 identified, Table 3. The highest *Enterobacteriaceae* count obtained was 5.30 log CFU/g which was observed in E.V market, and the lowest was 1.70 log CFU/g which was observed in Xipamanine market. The mean counts ranged from 1.89 to 4.39 log CFU/g. (Table 4).

Table 3
Distribution of *Enterobacteriaceae* bacterial strains identified on RTE lettuce salads vended in Maputo markets, Mozambique

No. of identified strains/Locations	**Museu**	**Povo**	**Mandela**	**Estrela Vermelha**	**Benfica**	**Xipamanine**	**Peixe**	**Total Individual Strains**	***% of individual strains***
Citrobacter freundii	0	0	2	0	0	0	1	3	**6**
Citrobacter koseri	0	0	0	0	0	1	0	1	**2**
Enterobacter asburiae	0	2	1	1	2	2	1	9	**18**
Enterobacter cloacae	1	2	3	4	4	1	2	17	**34**
Klebsiella oxytoca	0	1	0	1	0	0	1	3	**6**
Klebsiella pneumoniae	4	4	1	3	0	1	1	14	**28**
Kosakonia cowanii	0	0	0	1	0	0	0	1	**2**
Raoultella ornithinolytica	0	1	0	0	0	0	0	1	**2**
Raoultella terrígena	0	0	0	1	0	0	0	1	**2**
Total	5	10	7	11	6	5	6	50	**10** **0**
% of individual strains	**10**	**20**	**14**	**22**	**12**	**10**	**12**	**100**	

Table 4
Prevalence of *Enterobacteriaceae* on RTE lettuce salads vended in Maputo markets, Mozambique

Location	**Number of samples**	**% possitive**	**% unsatisfactory (≥ 4 Log CFU/g)**	**Mean Count (log CFU/g)**	**Min-Max**
Museu	5	100	20	3.18 ± 0.93	2.00 - 4.08
Povo	5	100	40	3.93 ± 0.28	3.49 - 4.19
Mandela	5	100	20	3.18 ± 0.93	2.00 - 4.08
Estrela Vermelha	5	100	80	4.39 ± 0.70	3.51 - 5.30
Benfica	5	100	0	3.15 ± 0.74	1.82 - 3.58
Xipamanine	5	100	0	2.80 ± 0.89	1.70 - 3.60
Peixe	5	60	20	1.89 ± 1.89	0.00 - 4.41

3.3 Total plate count and identification of *Enterobacteriaceae* on drinking water

Forty-two drinking water samples (35 from the manipulative reservoirs and 7 from the public supply) were analyzed in this research to see the prevalence of *Enterobacteriaceae*. All (n = 7) drinking water samples collected from the distribution source showed be negative for the *Enterobacteriaceae*. Thirty-one percent (n = 11) of the samples were found to be positive for *Enterobacteriaceae* of a total of 35 samples of manipulative reservoirs. The identified species included; *Enterobacter cloacae* (36.36%)*, Enterobacter asburiae* (27.27%)*, Klebsiella pneumonia* (18.18%) *Citrobacter freundii* and *Kluyvera ascobarta* with (9.09%), Table 5. The mean *Enterobacteriaceae* counts ranged from 0.24 to 2.01 log CFU/ml. The high count was registered in the market with 3.98 log CFU/ml and the lowest was recorded in the Povo market with 1.22 log CFU/ml. Notably all water samples collected from the Peixe market were negative, Table 6. Most (60%) of the samples from the Benfica and Xipamanine markets were positive for the presence of *Enterobacteriaceae.*

Table 5

Distribution of *Enterobacteriaceae* bacterial strains identified in drinking water used by vendors to clean salads in Maputo markets, Mozambique

Indentified strains/Location	***Museu***	***Povo***	***Mandela***	***Estrela Vermelha***	***Benfica***	***Xipamanine***	***Peixe***	***Total individual strains***	***Relative %***
Citrobacter freundii	0	0	0	0	1	0	0	1	9,09
Enterobacter asburiae	0	1	0	1	0	1	0	3	27,27
Enterobacter cloacae	1	0	0	1	0	2	0	4	36,36
Klebsiella pneumoniae	0	0	0	0	1	1	0	2	18,18
Kluyvera ascorbata	0	0	0	1	0	0	0	1	9,09
Total	1	1	0	3	2	4	0	11	100

Table 6

Prevalence of *Enterobacteriaceae* in drinking water used by vendors to clean salads in Maputo markets, Mozambique

Location	**Number of samples**	**% possitive**	**Mean Count (log CFU/g)**	**Min-Max**
Museu	5	20	0.48 ± 1.07	0.00 - 2.40
Povo	5	20	0.24 ± 0.55	0.00 - 1.22
Mandela	5	40	1.39 ± 1.90	0.00 - 3.56
Estrela Vermelha	5	20	0.48 ± 1.07	0.00 - 2.40
Benfica	5	60	2.01 ± 1.43	0.00 - 3.52
Xipamanine	5	60	1.57 ± 1.66	0.00 - 3.98
Peixe	5	0	0.00 ± 0.00	0.00 - 0.00

3.4. Total plate count and identification of *Enterobacteriaceae* on surfaces contact

In this study 105 swabs (35 hands; 35 bowls and 35 knifes) were collected. The *Enterobacteriaceae* isolate identities were determined using MALDI-TOF-MS analysis. The identified species include; *Enterobacter cloacae* (35.66%), *Klebsiella pneumonia* (20.93%), *Enterobacter asburiae* (11.63%), *Citrobacter freundii* (8.53%), *Klebsiella oxytoca* (6.98%), *Kluyvera ascobarta* (4.65%) and *Escherichia coli* (3.88%). Other species like *Citrobacter koseri, Kosakonia cowanii*, *Raoultella ornithinolytica* and *Raoultella terrigena* were found in were found in very low percentages, Table 7. Regarding the number of species of *Enterobacteriaceae* isolated, the E.V market was the one that registered the most with 29 isolates. On the other hand, the Xipamanine market was the one which recorded a smaller number of isolates (10). On surfaces (hands, knife and bowl), the counts of *Enterobacteriaceae* ranged from 2.18 to 4.48 log CFU/cm^2. In general, hand samples were the ones with the highest counts (2.18 - 4.48 log CFU/cm^2). The Benfica market recorded the highest (4.48 log CFU/cm^2) counts in all surfaces samples, Table 8. The Xipamanine Market recorded low mean counts of *Enterobacteriaceae*.

Table 7

Distribution of *Enterobacteriaceae* bacterial strains identified on hands surfaces used by vendors in Maputo markets, Mozambique

Indentified strains/Market Location	**Museu**	**Povo**	**Mandela**	**Estrela Vermelha**	**Benfica**	**Xipamanine**	**Peixe**	**Total Individual Strains**	**Relative %**
Citrobacter freundii	2	0	0	4	0	1	4	11	8,53
Enterobacter asburiae	0	2	2	1	4	0	6	15	11,63
Escherichia coli	2	2	0	1	0	0	0	5	3,88
Enterobacter cloacae	3	8	5	10	5	7	8	46	35,66
Kluyvera ascorbata	0	0	0	1	5	0	0	6	4,65
Klebsiella oxytoca	1	4	2	1	1	0	0	9	6,98
Kluyvera cryocrescens	0	0	1	0	0	0	0	1	0,78
Kluyvera intermedia	0	0	1	0	0	0	0	1	0,78
Kluyvera georgiana	1	0	0	0	0	0	0	1	0,78
Klebsiella pneumoniae	4	6	4	7	2	2	2	27	20,93
Kosakonia cowanii	0	1	1	0	0	0	0	2	1,55
Leclercia adecarboxylata	0	0	0	4	0	0	0	4	3,10
Raoultella ornithinolytica	0	0	0	0	1	0	0	1	0,78
Total	**13**	**23**	**16**	**29**	**18**	**10**	**20**	**129**	**100,00**

Table 8
Distribution of *Enterobacteriaceae* bacterial strains identified on drinking water used by vendors to wash salads in Maputo markets, Mozambique

Markets	Type of sample	Number of samples	% possitive	Mean Count (log CFU/g)	Min - Max
Museu	Hand	5	100	3.02 ± 0.36	2.65 - 3.49
	Knife	5	60	2.73 ± 0.64	0.00 - 3.18
	Bowl	5	80	3.31 ± 0.09	0.00 - 3.35
Povo	Hand	5	100	3.23 ± 0.64	2.18 - 3.88
	Knife	5	100	2.67 ± 0.29	2.40 - 3.06
	Bowl	5	100	3.08 ± 0.41	2.60 - 3.64
Mandela	Hand	5	100	3.23 ± 0.41	2.65 - 3.72
	Knife	5	80	2.90 ± 0.27	0.00 - 3.18
	Bowl	5	80	3.03 ± 0.38	0.00 - 3.31
Estrela Vermelha	Hand	5	100	3.69 ± 0.15	3.16 -3.86
	Knife	5	80	3.43 ± 0.20	0.00 - 3.62
	Bowl	5	100	3.44 ± 0.91	2.18 - 4.16
Benfica	Hand	5	80	3.75 ± 0.92	0.00 - 4.48
	Knife	5	80	2.88 ± 1.24	0.00 - 4.48
	Bowl	5	100	2.99 ± 0.96	2.18 - 4.48
Xipamanine	Hand	5	80	2.29 ± 0.90	0.00 - 3.60
	Knife	5	80	2.29 ± 0.90	0.00 - 3.60
	Bowl	5	80	2.67 ± 0.82	0.00 - 3.45
Peixe	Hand	5	100	4.27 ± 0.11	4.14 - 4.41
	Knife	5	60	2.77 ± 0.11	0.00 - 2.88
	Bowl	5	100	3.05 ± 0.27	2.65 - 3.34

3.5 Prevalence of E. coli in the sample

No E. coli was isolated from the potable water (n = 42) and the salad lettuce (n = 35) samples. On the other hand 5/182 samples were positive for E. coli isolates and it were isolated from bowls samples.

3.6 Virulence genes in *E.coli* isolates

The PCR assay was used for conforming the presence of virulence genes in *E.coli* but the virulence genes mdhF, mdhR, stx1F, stx1R, stx2F, stx2R, were not detected in any of the *E.coli* isolates.

3.7 Correlation among the *Enterobacteriacea*e counts in the RTE lettuce salads, drinking water and surfaces

A summary of the correlation for the samples (RTE lettuce salads, drinking water and surfaces) is shown in Table 9. There was not a significant positive correlation found between the counts founded in RTE lettuce salads and

drinking water, and the counts founded in RTE lettuce salads, hands and surfaces, and there were no significant differences in *Enterobacteriaceae* counts of swabs samples between the locations (P>0.05).

Table 9
Correlation between the counts of *Enterobacteriacea*e founded in the samples

Level	Spearman's rho	Sig.*
Salads – water	0.410*	0.000
Salads – hand	0.199	0.000
Salads – knife	0.074	0.000
Salads – bowl	0.223	0.000
Water – hand	0.072	0.000
Water – knife	0.164	0.000
Water – bowl	0.337*	0.000
Hand – knife	0.218	0.000
Hand – bowl	0.276	0.000
Knife – Bowl	0.281	0.000

*correlation is significant at the 0.01 level (2 – tailed)

4. Discussion

4.1 *Enterobacteriacea*e founded

In the current study, we evaluated the prevalence of *Enterobacteriaceae* from ready-to-eat salads, drinking water and swabs in markets of Maputo by direct Matrix Assisted Laser Desorption/Ionization Mass Spectrometric – (MALDI-TOF-MS). The predominant species of *Enterobacteriacea*e found were: *Enterobacter cloacae* (34%), *Klebsiella pneumonia* (28%), *Enterobacter asburiae* (18%), *Citrobacter freundii* and *Klebsiella oxytoca* (6%). *E. coli* as one of the important public health strains were also found but in low counts. *Enterobacter* spp. in 18%, *Klebsiella oxytoca* in 8%, and *Escherichia coli* were not isolated in any of the samples. Recently, Shiningeni et al., reported high (83%) percentages of *Enterobacteriacea*e in RTE food vended in Windhoek, Namibia [41].

The *Enterobacteriacea*e family is a part of the normal gut microbiota but can also be found in the environment [16,42,43]. *Enterobacter*, *Citrobacter* and *Klebsiella* species are the mostly found in environments. For instance, water, salads, hands and utensils can be contaminated with these microorganisms. Many of the bacterial strains of *Enterobacteriacea*e family, are used to be dismissed as harmless commensals and usually considered by food manufacturers as hygiene indicators and therefore used to monitor the effectiveness of implemented preventive pre-requisite measures such as Good Manufacturing Practices and Good Hygiene Practices [14,44]. The presence of low levels of *Enterobacteriaceae* in foods is accepted and does not represent a direct safety concern. Members of the *Enterobacteriacea*e family are opportunistic pathogens

responsible for a major health problems worldwide [45,46,47,48]. The genera *Escherichia, Klebsiella, Enterobacter* ,as *Serratia,* and *Citrobacter* have been reported to be responsible for infections in humans and other animals. *Citrobacter* species are an uncommon cause of bacterial meningitis in neonates, but are associated with brain abscesses in the majority of cases [49]. *Klebsiella* species and *E. coli* can become carbapenem-resistant. *Klebsiella pneumoniae,* is responsible for pneumonia [50], and represent highest risk at the patients those with impaired immune systems, [51]. The important foodborne pathogens that are found in the *Enterobacteriaceae* family include Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Shigella spp., *Salmonella* (non-typhoid), *Salmonella* (Typhi/Paratyphi), *Yersinia enterocolitica* and Cronobacter spp [52]. In our study we did not found these pathogens foodborne.

4.2 Prevalence of *Enterobacteriaceae in* RTE lettuce salads

The mean *Enterobacteriaceae* ranged from ranged from 1.89 ± 1.89log CFU/g to 4.39 ± 0.70 log CFU/g. The highest mean was observed in E.V market and the lowest mean in Peixe market. The highest count level was 5.30 log CFU/g and was observed in E.V Market. Similar results were found in Rwanda, investigated kitchen scale salad preparation practices in a field study (food service establishments) [53]. Unsatisfactory levels of *Enterobacteriaceae* ((≥ 4 Log CFU/g) (ICMSF) were detected in 25.7% (9/35) RTE lettuce salads samples tested. Unsatisfactory levels of *Enterobacteriaceae* were the highest in E.V with prevalence of 80% (table 4). The presence of the highest level of *Enterobacteriaceae* is indicative of unacceptable contamination during food preparation and inappropriate conditions such as prolonged storage at elevated temperature [44,54]. These findings are comparable with other studies done worldwide. In Namibia the highest mean counts were 4.10 log CFU/g. In Ruanda the highest mean *Enterobacteriaceae* count was 3.3 log CFU/g and in Zambia and Mashhad the counts ranged between 1.6 to 9.8 log CFU/g [55,41,53]. Although other authors, studied the prevalence *Enterobacteriacea* in fresh vegetables sold in retail of Canada over a period of four years (2009 – 2013), and found counts generally very low, with prevalence intervals ranging from 0 - 1.3 log CFU/g [56]. Unsanitary vending conditions, unhygienic practices act, insufficient food hygiene education and presence of reservoirs and vectors in or near the food production or service areas can contribute to increase the level of contamination of ready-to-eat foods [49], and it can be associated with the results.

The dominant identified species in this study included *Enterobacter cloacae, Klebsiella pneumonia* and *Enterobacter asburiae,* and can indicate poor food

preparation, poor sanitary conditions as well as Also cross contamination. These species are genetically related bacteria used to assess the general hygiene status of a food product. These microbes can be introduced in food with cross contamination especially RTE salads. Recently authors, demonstrated the occurrence of various microbial pathogens which includes *Escherichia coli* in ready to eat vegetable salads in developing countries [1]. However, in this study *E. coli* were not detected in ready-to-eat lettuce salads, contrasting with other studies in other African countries like Namibia, Ghana and India, with where those bacteria were found in highest levels [13,57,41].

Although, the absence counts of *E. coli* isolates in RTE lettuce salads, we cannot ensure that this bacteria is not in the salads, as generally known *E.coli* is part of the normal microbiota in the digestive tract of both humans and animals [58]. This bacterium can be secreted, often in large numbers, through the feces into the environment [16]. The absence may be due the fact that the samples were collected during the dry season (no rain). Among the isolated, we cannot ignore the high counts obtained for the coliform bacteria such as *Enterobacter* spp, *Citrobacter* spp, *Klebsiella* spp been increasingly reported as important opportunistic pathogens [59].

The microbiological quality and safety of RTE lettuce salads sold in markets can be compromised at numerous points along a food system from farm to consumption. Since there is no step that kills pathogens during the production of RTE salads, a completely safe final product can never be guaranteed. In this perspective measures to reduce the contamination might be advised such as a proper handling and washing before consumption of these products as well as public education and awareness. Appropriate irrigation water is also important [16,60].

4.1 Prevalence of *Enterobacteriaceae in* drinking water

Tests for *Enterobacteriaceae* bacteria, as indicator of hygiene quality were done in 42 drinking water samples. It is well documented that fecal contamination of drinking water can cause numerous disease outbreaks [61]. In this study, *E. coli* was not enumerated from any of the drinking water samples indicating that that the water has not been contaminated with feces. Different results have been found in developing countries such as Kenya, India and Iran on what *E. coli* were found in 30%, 30% and 61% in drinking water respectively [62,63,64]. *Acinetobacter, Aeromonas, Enterobacter sakazakii, Helicobacter pylori, Klebsiella, Pseudomonas aeruginosa* bacteria, provides information on organisms that have been suggested as possible causes of waterborne disease [51]. In this study were not found all of this bacteria group. *Klebsiella pneumoniae* is a part of the group that was most isolated. According to the WHO, ideally, drinking-water should be free from known pathogenic micro-organisms capable

of causing disease or any bacteria indicative of fecal contamination [65,51,66]. Although no isolates of *E. coli* were found in the drinking water samples, other *Enterobacteriaceae* species were found in 31% (11 of 35) of the sample, which means that almost half of the seller's reservoir water samples were contaminated. This may compromise the health of consumers seeking markets to meet their food needs because a lack of access to safe drinking water can lead to various health problems [67].

Waterborne diseases represent a major human health risk in many parts of the world, especially in developing countries. Mozambique as a developing country is well known for persistent and recurrent waterborne diseases [68,69]. The availability of safe drinking water in developing countries remains a major challenge due to poor sanitation condition [70]. Safe sanitation is essential for health, from infection prevention to improving and maintaining mental and social welfare [71].

4.3 Prevalence of *Enterobacteriaceae in* swabs

The purpose of collecting swabs samples is to trace the sources of and evaluate the extent of the contamination [9]. In this study, the identified species included *Enterobacter* spp, *Citrobacter* spp, *Klebsiella* spp. and *E. coli. The* counts ranged from 2.01 to 4.39log CFU/g in the samples. The lowest (2.18log CFU/m^2) counts were observed in Peixe market and the highest (4.39 log CFU/m^2) were observed in E.V market. These findings concur with other study [72] in which they found 44% of *Enterobacteriaceae* in hands of food handlers. Other study founded *C. sakazakii* from 26.9% of 78 domestic kitchens visited in United States [73].

The species of *Enterobacteriaceae* identified in this study are responsible for cross-contamination and could signify unhygienic conditions during food handling and preparation [54]. From 105 swabs collected, five (5/105), isolates of *E.coli* were recovered. These findings concur with a study conducted in Zahedan who found total coliform and *E. coli* in dishes (86. 67%, and 33. 3%) and spoons and forks, (79% and 30%) in establishments [74].

In fact, food contact equipment is an important factor of microbial contamination of ready-to-eat products such as lettuce. Microorganisms can be transferred during food preparation such as cutting and grinding, specifically when the same equipment is used for raw material, meat and RTE foods [75]. Food handlers with poor personal hygiene could be potential sources of infection due to pathogenic bacteria [76], and can be a source of foodborne contamination and they can cross-contaminate raw and processed food stuffs [9,77]. Developing countries have high problems of food borne diseases [78], due to the difficulties in adopting optimal hygienic practices during food

handling [79]. In this study we did not find *E. coli* isolates in samples from the food-handlers, despite that, we cannot conclude that the hands of the handlers are safe. Food handlers may constitute a reservoir of virulent strains of *Staphylococcus aureus* and may be vehicles of their transmission to food [80,81]. The presence of the *E. coli* in the utensils is probably due to the fact that the utensils have not been washed properly. Not only that, but contamination may be due to the presence of flies that are prevalent in these unclean environments. On the other hand, high contamination of the hands may be related to improper hand washing and disinfection. Since food retailers in the markets do not have water pipes, the sellers store water in containers. The contamination could also be attributed to substandard cutting and preparation practices, particularly poor hygienic conditions of the premises that may result from rubbish, sewage and other noxious substances present in the vicinity

4.4 Virulence genes in *E.coli* isolates

Several studies have focused on determining the virulence genes in *E.coli* isolates of fresh produce sold at open hair markets, supermarkets and street venders from selected areas in a specific country [82,83,84,85], because it has been recognized as the leading causes of human food born infections throughout the world with fatal complications such as hemolytic uremic syndrome that ends in renal failure. The real-time PCR assay used for pathogen detection confirmed that the isolates of *E.coli* obtained using MALDI-TOF analysis were positive but the virulence genes stx1F, stx1R, stx2F, stx2R, were not detected in any of the *E.coli* isolates. These findings concur with other studies who collected vegetable salads samples from restaurants and market respectively and *E. coli* O157:H7was not detected in any of the samples analyzed [86]. In contrast *Escherichia coli* O157:H7 and *Listeria monocytogenes* were founded in different salad vegetables [87]. The negative results for the virulence genes of *E. coli* in the samples especially in the RTE lettuce salads can be explained by the fact that salt and vinegar are used to temper the salads in Mozambique. Acetic acid alone or combined with salt can inhibiting *Escherichia coli* O157:H7 for example [88,89,90]. Besides, in Mozambique, farmers do not use organic fertilizers basically, cattle and sheep are the major animal reservoir of STEC. Furthermore, we cannot ensure that enteric pathogens are not present as the survival and growth characteristics of different strains of *E. coli* and enteric pathogens can vary, [58]. Further studies are required to cover more numbers of samples and to investigate the presence of non shiga-toxin producing *E. coli*.

5. Conclusion

This study found high *Enterobacteriaceae* counts in RTE lettuce salads and other samples (what other sample, please list them) that could serve as an indicator for the need to promote improvement in sanitary and good hygienic practices in food markets of Maputo. *E. coli* was isolated in 4.76% of the surfaces samples and non-virulence's genes were found. However, we cannot disregard the importance of the *Enterobacteriaceae* isolates since some of them are used to indicate the sanitary conditions and many of them become pathogenic due to the acquisition of virulence-associated genes. The results are little evidence that those salads represent an important risk for transmission of pathogenic microorganism in in Maputo, Mozambique, and it can be a potential hazard for public health. Proactive research to ensure food processing in particular salads and hygiene controls are needed in Maputo markets to ensure food safety and preserve consumer health.

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8. Conflicts of interest

The authors declare that there are no conflicts of interests.

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4.4 Artigo 4

Artigo a ser submetido à revista *Journal of Food Safety*

Detection of virulence genes of *Escherichia* spp isolated from lettuce (*Lactuca sativa*) and ready-to-eat lettuce salads vended in Maputo food markets

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Abstract

Outbreaks of food origin attributed to the contamination of fresh products in particular, green leafy vegetables such as lettuce with Escherichia coli virulence genes are a growing concern. The aim of this study was to evaluate the microbial quality and the presence of *Escherichia coli* generic and virulence genes (stx1F, stx1R, stx2F, stx2R) in two different sample matrices (lettuce and ready-to-eat lettuce salad) vended in six food markets of Maputo. A total 70 samples (35 lettuces and 35 ready-to-eat lettuce salads) were collected and analyzed using standard plating techniques. A total of 27 and 31 isolated were identified in the lettuce and ready-to-eat lettuce salads in five markets of Maputo City, respectively. *Enterobacter asburiae* were obtained in 26% and 13%, *Enterobacter cloacae* in 22% and 16%, *Klebsiella oxytoca* were obtained in 22% and 6%, *Escherichia hermannii* in 4% and 10% and *Escherichia coli* in 0% and 6% in lettuce and ready-to-eat lettuce salads respectively. Lettuce salads ready for consumption were more contaminated than the lettuce (raw material). No virulence genes (stx1F, stx1R, stx2F, stx2R) were detected by qualitative polymerase chain reaction (qPCR) in Escherichia spp. species identified in

ready-to-eat salads. This study shows the importance of vegetables and salads as potential sources of *E. coli* infection.

1. Introduction

Consumption of fresh vegetables outside homes is increasing globally, necessitated by busy life schedules as well as nutritional and health advantages (FDA, 2018; Inyinbor, Bello, Oluyori, Inyinbor, & Fadiji, 2019; Mir et al., 2018). However, most of the food-borne outbreaks are associated with consumption of vended food in open markets (FAO/WHO, 2008; Faour-Klingbeil, Todd, & Kuri, 2016; Kyere et al., 2019; Mbae, Ndwiga, & Kiruki, 2018). Many studies have reported the occurrence of various microbial pathogens such as *Escherichia coli*, *Listeria monocytogene*s, Salmonella in ready-to-eat vegetable salads (Kundu, Wuertz, & Smith, 2018; Mir et al., 2018; Oliveira, Abadias, Colás-Medà, Usall, & Viñas, 2015; Ponniah et al., 2010).

The Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) investigated several multistate foodborne disease outbreaks involving vegetables, salad mix, in the past three years (2016-2018). Norovirus, Salmonella and *E. coli* were implicated as the main etiological agents of the foodborne disease outbreaks (Johnson, 2019).

A study by the United Nations Food and Agriculture Organization and the World Health Organization reported that out of a total of 957 outbreaks identified due to pathogenic *Escherichia coli* (*E. coli*), in 27 different countries, 15% was attributed to vegetables and fruit (FAO/WHO, 2018). The microbiologically contaminated fresh vegetables represent a constant threat to public health and a significant risk for consumers, especially vegetables without thermal processing (FAO/WHO, 2018; Kljujev et al., 2018). Similarly, Berrada et al. (2016) reported that raw, cooked and fruits salads were contaminated by the total aerobic mesophilic flora, total coliform and *E. coli*.

Escherichia coli, is a large and diverse group of bacteria commonly found in the environment, foods, gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless and are an important part of a healthy human intestinal tract. However, some are pathogenic and can cause illness, either diarrhea or illness outside of the intestinal tract (urinary tract infections, respiratory illness, pneumonia,) because have acquired virulence genes.

There are at least five distinct diarrheagenic *E. coli* categories or pathotypes which include Enterohemorrhagic, Enterotoxigenic, Enteroinvasive, Enteropathogenic and Enteroaggregative. These types of *E. coli* can be transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables (CDC).

Several studies have focused on determining the virulence genes in *E.coli* isolates of fresh produce sold at open air markets, supermarkets and street vendors from selected areas worldwide (Dinu & Bach, 2013; Seow, ágoston, Phua, & Yuk, 2012; Tzschoppe, Martin, & Beutin, 2012). Leafy vegetables have been implicated in several outbreaks of foodborne disease caused by E. coli O157:H7, a pathogen of increasing public health significance (Delaquis, Bach, & Dinu, 2007; Osés et al., 2010).

The Foodborne Disease Load Epidemiology Reference Group estimated that each year food-borne STEC infections, as opposed to water, person-to-person contact or other routes transmission has caused over one million diseases and 128 deaths annually, in developing countries (FAO/WHO, 2018). The contamination of fresh vegetables may occur at all stages during production and processing (Lehto, Kuisma, Määttä, Kymäläinen, & Mäki, 2011). Water has been recognized as an important microbial risk factor for vegetables in many production areas (Decol et al., 2017). Irrigation ground water influences the microbiological quality of ready-to-eat salads as it can be a source of E. coli that may enter the food chain through vegetable ingestion. Several authors have shown a close connection between the level of water contamination used for irrigation and the level of contamination of lettuces (Araújo et al., 2017; Decol et al., 2017; Rock et al., 2019). For example, *E. coli* were isolated from leafy greens vegetables vegetables and irrigation water in different production systems in South Africa (Jongman and Korsten, 2017).

Working conditions and practices of food handlers also influence on the microbiological quality of fresh-cut lettuce salads (Pereira, Rodrigues, & Ramalhosa, 2013). Washing fresh vegetables with or without sanitizers is one of the important steps designated to reduce or eliminate microbial hazards in fresh vegetables (Ssemanda et al., 2017).

Food markets have become important sources of affordable food for many people; however, these markets have been associated with major outbreaks of foodborne illness (WHO, 2006). Studies on microbiological quality and virulence genes in bacterial isolated from lettuce and ready-to-eat lettuce salads provided by vendors in markets of Maputo, Mozambique are limited. Therefore, this study aims to determining the microbiological quality and virulence genes in E. coli bacterial isolates from lettuce and ready-to-eat lettuce salads vended in food markets in Maputo, south Mozambique.

2. Materials and methods

2.1. Sample collection

A total of seventy samples (35 lettuces and 35) ready-to-eat lettuce salads) were collected from each of the five markets in Maputo, Mozambique, over a 3-month period from August to October 2019. To obtain a representative sample for each market, random samples were collected in different points of the markets. Typically food markets in Maputo -Mozambique, are open-air, made up of small establishments or small food service called “stalls.” The samples selected are common dishes for breakfast and lunch. Samples from each vendor consisted of lettuce and a ready-to-eat lettuce salad. At least, 100 g samples were collected from 7 vendors in each market in sterile plastic bags kept in ice chest, maintained at 0–4 °C and taken to the Laboratory of Microbiology and Safety of the University Edurado Modlane, Maputo campus, Mozambique and processed within 2–4 h for microbial analysis.

2.2. Detection E. coli in the samples

The prevalence of E. coli in the samples was detected by following the method described in Bacteriological Analytical Manual, 1998. The samples were processed in the laboratory immediately upon receipt. The selective and differential media Eosin Methylene Blue Agar (EMBA, Oxoid) were used. Briefly a 25 g sample from each sample was stomached using Stomacher®-400 (Seward, Norfolk, UK) for 2 min at 230 rpm in 225 ml of 0.1% peptone water. All the samples were enrichment (incubation at 37 °C for 6 h). After that 0.1 ml of initial base solution were transferred to EMBA plates and incubated at 37 °C for

24 ± 2h. After incubation, presence of typical small gray colonies, 1.5–2 mm in diameter, with central depression and black zone around were considered as Escherichia colonies. The presumptive E. coli were picked for further confirmation.

2.3. Confirmation of presumptive isolates colonies using MALDI-TOF-MS

All bacterial isolates were confirmed using matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF-MS). Purified bacterial cultures stored on Nutrient Agar media (manufacturer) were transferred directly to MALDI-TOF steel polished target plate (Bruker, Bremen, Germany) and overlaid with the cyano-4-hydroxycinnamic acid matrix (Bruker). The target plate was subsequently analyzed using MicroFlex LT MALDI-TOF-MS (broker) in conjunction with Biotyper automation software and libray (Bruker). Duplicate score values were recorded and used to determine the accuracy of identification. The MALDI-TOF-MS test was carried at the Centre of Excellence in Food Security, Department of Plant and Soil Sciences, University of Pretoria.

2.4 Identification of virulence genes in the confirmative Escherichia spp.

Virulence genes (mdf, mdhR, stx1F, stx1R, stx2F, stx2R) in Escherichia spp. were detected using the polymerase chain reaction (PCR). Total genomic DNA was extracted from pure cultures using Zymo Kit – Quick DNA Minipre Kit following the manufacturer's protocol recommendation. The virulence genes were amplified using specific primers. Amplification reactions were performed in a total volume 25 µl of PCR reaction volume. Amplification reactions were carried out in (c1000 Touch) thermocycler at the Centre of Excellence in Food Security, Department of Plant and Soil Sciences - Pretoria University. Amplification of the expected band size signifies the presence of virulence genes.

2.5. Statistical analysis

All information regarding the microbiological quality of the sample was collected and recorded in the Microsoft Excel worksheet (version 2010). The statistical analysis of data was performed using the Statistical Package for

Social Sciences (SPSS, version 21.0 Inc. Chicago, IL, USA). We used Microsoft Access 2003 (Microsoft, Redmond, WA) for data entry and SPSS 12 for Windows (SPSS, Chicago, IL) for data analysis. Multivariable logistic regression models were conducted to determine and evaluate the significance of differences in prevalence of E.coli in lettuce and ready-to eat-salads samples.

3. Results and discussion

3.1. Microbiological quality of lettuce and ready-to-eat lettuce salads

A total of 27 and 31 isolates of presuntive Escherichia coli were obtained from lettuce and ready to eat lettuce salad respectively using MALDI-TOF-MS (Table 1). The most isolates included Enterobacter asburiae and Enterobacter cloacae (19%), Klebsiella oxytoca (14%), Klebsiella pneumoniae (10%) (Figure 1) E. coli was not presumptively isolated from lettuce, however, in ready-to-eat salad lettuce samples, Escherichia genera were the most frequently isolated with the prevalence of 17.14% (6/35) for E. coli and 8.57% (3/35) for Escherichia hermannii.

These results are similar to those obtained by Nousiainen et al. (2016) whereupon E. coli was isolated from 15% of the samples. The fact that almost all of our lettuce and ready–to-eat salad samples are contaminated with Enterobacteriaceae including E. coli, regardless of location, is indicative of a possible association with primary contamination, that is, even before lettuces reach the markets during the moment of cultivation, they are already subject to contamination. In fact, both the large surface, which easily comes into contact with the soil and irrigation water, facilitates the accumulation of dirt and the adhesion of bacteria (Zwe & Yuk, 2017). However, we cannot say that the source of the contamination was the place of cultivation, since the contamination may also have its origin in manipulation or else it may result from cross-contamination.

Thus, contamination can also likely it originated from poor sanitation in the Maputo markets as well as from poor hygiene practices. Additionally, in Mozambique there is still the worst scenario in which fresh salads, especially lettuce, are consumed without going through the sanitation process. As know, vegetables are usually consumed raw; consequently, there is a need of best hygienic conditions from the harvest to the processing because of the gastro-

enteritis that they could provoke (Coulibaly-Kalpy, Adouko Agbo, Adjehi Dadie, & Dosso, 2017). Microbial pathogens can be easily introduced and reside for extended periods in wash baths, favoring cross contamination until the final products (Holvoet et al., 2014). This may be the likely explanation for the presence of E.coli in the ready-to-eat lettuce salad samples, as there is a vulnerability of fresh produce to cross contamination during the washing phase without the use of sanitizers regardless of drinking water use. As is common knowledge water management in fresh produce processing is an important factor affecting the microbial quality and safety of fresh produce (Holvoet et al., 2014).

According Faour-Klingbeil, Kuri and Todd (2015) in developing countries, inaccessibility to safe water, lack of agricultural infrastructures and limitations to implementing good agricultural practices (GAP) are persistent challenges. In addition, the contamination may have originated through the utensils used during the preparation of food, as well as the hands of the handlers, since it was possible to observe during the research that some vendors used their hands to mix the salads.

This study shows that independently of the markets overall hygienic status, most ready-to-eat salads from Maputo-City had a poor microbiological quality. Although E. coli were not found in the lettuce samples we cannot ignore the other isolates as they belong to the Enterobacteriaceae members. As is well known, of which are widely distributed in the environment, includes many important human pathogens (Takahashi et al., 2017) and since ready-to-eat foods are edible without additional treatment, risks of foodborne outbreaks may be high (Coniglio, Faro, & Marranzano, 2016).

It is officially prohibited to irrigate leafy vegetables (lettuce, spinach, coriander) with untreated sewage. However, this practice is common in Vale do Infulene, where fresh vegetables are eaten in the city of Maputo.

Our results provide some microbiological quality indices of lettuce and ready-to-eat salads sold in the Maputo markets, because it is known the contamination of fresh vegetables and consequently the contamination of ready-to-eat lettuce salad may occur at all stages during production (Lehto et al., 2011). Working conditions and practices of food handlers also influence on the microbiological quality of fresh-cut lettuce salads (Pereira et al., 2013). To

avoid contamination of ready-to-eat salads, vendors should sanitize the raw material during washing and adopt good practices.

As known, washing fresh vegetables with or without sanitizers is one of the important steps designated to reduce or eliminate microbial hazards in fresh vegetables (Ssemanda et al., 2017). The higher prevalence of E. coli and coliform in the samples indicates the need for improved monitoring of fresh produce in the markets and public education for the importance of washing produce before consumption. Government entities should also be involved, ensuring that hygiene and environmental conditions are controlled and maintained in the markets.

Table 1
Identified bacteria in the lettuce and ready-to-eat lettuce salads in five markets of Maputo City

Number of identified strains	Lettuce	Ready-to-eat Lettuce salads	Lettuce %	Ready-to-eat Lettuce salads %	Total	Total %
Citrobacter freundii	2	4	7	13	6	10
Enterobacter asburiae	7	4	26	13	11	19
Enterobacter cloacae	6	5	22	16	11	19
Escherichia coli	0	6	0	19	6	10
Escherichia hermannii	1	3	4	10	4	7
Klebsiella oxytoca	6	2	22	6	8	14
Klebsiella pneumoniae	3	4	11	13	7	12
Kluyvera ascorbata	2	0	7	0	2	3
Raoultella ornithinolytica	0	2	0	6	2	3
Raoultella planticola	0	1	0	3	1	2
Total	**27**	**31**	**100**	**100**	**58**	**100**

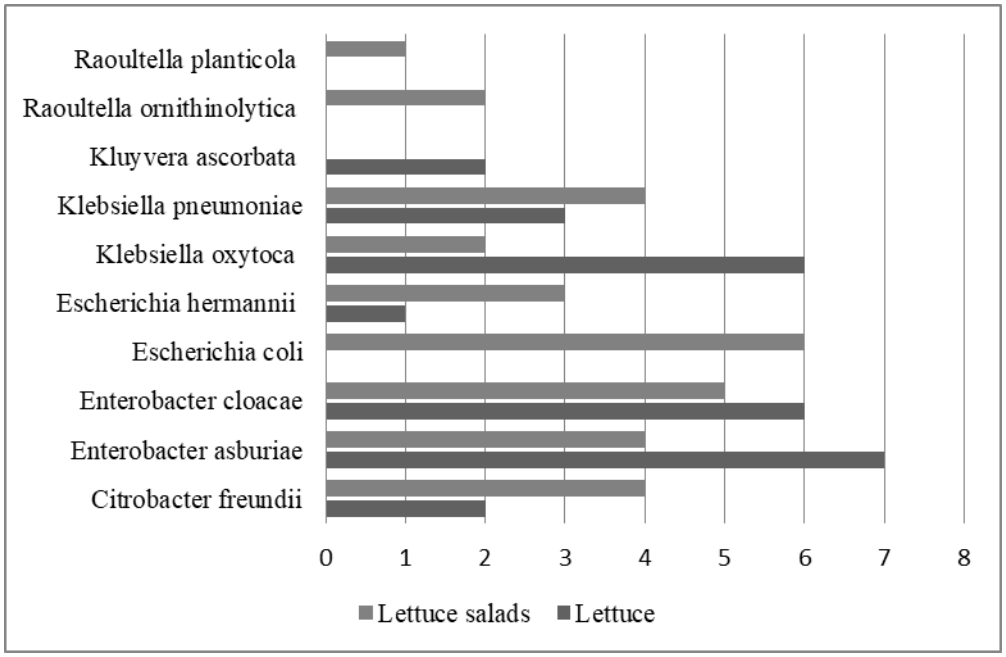

Figure 1: Comparison between isolated species of microorganisms in lettuce and ready-to-eat lettuce salads samples in the five markets of Maputo City visited

3.2 Identification of virulence genes in the E.coli isolates from the Ready-to-eat lettuce salads in markets of Maputo city

In this study, quantitative PCR (qPCR) were used to detect virulence genes along the ready-to-eat lettuce salads. The real-time PCR assay used for pathogen detection confirmed that the six isolates of E. coli and three E. hermannii were positive but the virulence genes stx1F, stx1R, stx2F, stx2R were not detected. These findings concur with other studies that collected vegetable salads samples from supermarkets in Turkey and retail in Vancouver, British Columbia which none of the samples of vegetable and salads samples was positive for virulence genes (Kemal Buyukunal, 2015; Wood, Chen, Friesen, Delaquis, & Allen, 2015). In contrast, Shakerian, Rahimi and Emad (2016) founded that 130 (87%) of 149 E. coli isolates from vegetable salads, sold in restaurants were STEC positive. Consumption of ready-to-eat salads has increased worldwide. Consequently, the number of outbreaks caused by food-borne pathogens, including diarrheagenic E. coli pathotypes (DEPs), associated with the consumption of ready-to-eat salads has increased (Castro-Rosas et al., 2012). Toe at al. (2018) founded 35.3% of E. coli with virulence genes in a total of 436 strains of E. coli isolated from 306 ready-to-eat raw mixed vegetables salads sold in collective catering in Abigjan. Additionally,

Tzschoppe et al., (2012) founded enterohemorrhagic E. coli isolates in 12.5% of 567 ready-to-eat vegetables. This proves that in fact fresh vegetables eaten can be a source of contamination of pathogenic bacteria and can be a danger to public health.

The negative results for the virulence genes of E. coli in the samples analysed in this study can be explained by the fact that, in Mozambique, farmers do not use organic fertilizers basically; cattle and sheep are the major animal reservoir of STEC. Although no virulence genes of Escherichia was detected in ready-to-eat salads samples analyzed in this study, high prevalence bacterial imply that effective control measures should be implemented to improve the microbiological quality of fresh produce sold in markets of Maputo.

4. Conclusions and recommendations

The results of this study revealed that the bacteria lettuces and lettuce salads ready for consumption sold in the markets of the city of Maputo showed a variability in the contamination of bacteria belonging to the Enterobacteriaceae family. The isolates found were Enterobacter asburiae (26% and 13%), Enterobacter cloacae in (22% and 16%), Klebsiella oxytoca (22% and 6%), Escherichia hermannii (4% and 10%) and Escherichia coli (0% and 6%) in lettuce and ready-to-eat lettuce salads respectively. Lettuce salads ready for consumption were more contaminated than the lettuce (raw material). No virulence genes (stx1F, stx1R, stx2F, stx2R) were detected by qualitative polymerase chain reaction (qPCR) in Escherichia spp. species identified. This study shows the importance of vegetables and salads as potential sources of E. coli infection and the importance of detecting virulence genes in fresh products before pointing out the water used for irrigation as a source of contamination. Studies are needed to cover more sample numbers and to investigate the presence of foodborne pathogens, especially shiga toxin-producing E. coli. In addition, it is important that, in the future, the relationship between the microbiological quality of the water used in the irrigation of fresh vegetables and the microbiological quality of ready-to-eat salads sold in the markets of Maputo city be studied, and establish a relationship between the levels of contamination that will be found. This study confirms the need to implement strategies to

increase the microbial safety of fresh products sold in the markets of Maputo, southern Mozambique.

5. Conflicts of interest

The authors declare no conflict of interest.

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5. DISCUSSÃO GERAL

O consumo de alimentos fora de casa é cada vez maior, sendo que o número de surtos alimentares associados a esses produtos também tem aumentado mundialmente. Os serviços de alimentação estão entre os principais locais de ocorrência de surtos alimentares mundialmente (MU 2020; BRASIL, 2017; ESTADOS UNIDOS, 2016).

Em Moçambique, os serviços de alimentação são, na sua maioria, pequenos estabelecimentos encontrados nos mercados conhecidos localmente por "baracas" e vendedores ambulantes. Os restaurantes existem, entretanto são em pequena quanidade e também são frequentados por um número pequeno de pessoas. As principais refeições servidas nestes locais são o pequeno almoço (café da manhã) e o almoço, e as preparações são constituídas maioritariamente por arroz, um ensopado de carne ou carnes assadas e saladas de alface.

No primeiro artigo da presente Tese, o qual avaliou às condições higiênico-sanitárias de preparo de alimentos nos serviços de alimentação em mercados públicos da cidade de Maputo, foi verificado que os manipuladores de alimentos avaliados eram na sua maioria mulheres (92,7%) jovens e todas nunca tinham sido treinadas em assuntos relativos à segurança de alimentos. Este achado vai ao encontro ao relatado nos estudos de Kibret & Abera (2012) e Rahman et al. (2012) que demonstraram que a maioria dos vendedores de alimentos que operavam nos mercados da cidade Bair Dar, na Etipia, eram mulheres jovens. Estes resultados podem ser explicados pelo fato de as mulheres serem geralmente responsáveis por preparar e cozinhar refeições em todo o mundo. Além disso, a venda de alimentos prontos para consumo em estabelecimentos públicos é uma atividade que não requer um treinamento complicado ou investimentos significativos, servindo de fonte de trabalho e renda para pessoas desempregadas, as quais vivem em áreas urbanas (ALLEN; SACHS, 2007; GRANT, 2012).

Outro fato importante a ser citado é que nos mercados avaliados havia acesso à água potável de rede, no entanto, não se pôde confirmar a potabilidade da mesma nas barracas, pois a água utilizada pelos vendedores

era armazenada em recipientes plásticos, em cada estabelecimento, e esses recepientes poucas vezes ou nunca eram higienizados (estavam visivilmente sujos). A conservação da água em recipientes plásticos devia-se ao fato da existência de apenas um único ponto de distribuição de água potável em cada mercado, obrigando que os manipuladores de cada serviço de alimentação coletassem e transportassem a água, diariamente. Essa atividade, além de inadequada dos pontos de vistas práticos e higiênico-sanitário, também não é adequada pelos pontos de vistas ergonômicos e de saúde dos manipuladores, os quais são obrigados a transportar recipientes bastante pesados, por distâncias consideráveis.

Além disso, não era feito um controle periódíco para conhecer a qualidade microbiológica da água armazenada nesses recipientes. Apesar do regulamento moçambicano não exigir o monitoramento periódico da água utilizada para a preparação dos alimentos nos serviços de alimentação, regulamentos mais recentes como é o caso da RDC n° 216/2004, no Brasil, estabelece que deve-se realizar um controle periódico da água potável utilizada para a preparação dos alimentos nos serviços de alimentação e que a higienização do reservatório de água deve ser realizada pelo menos de seis em seis meses (BRASIL, 2004).

Dos 191 estabelecimentos estudados, 85,76% apresentaram um Índice de Segurança de Alimentos (FSI) entre 0,15 e 0,18, sendo, portanto, classificados como inadequados quanto às condições higiênico-sanitárias. Escassez de água potável nos estabelecimentos para a preparação de alimentos, falta de instalações adequadas para preparação de alimentos, conservação dos alimentos em temperaturas inadequadas, após o seu preparo foram os principais fatores que contribuíram para essa inadequação. Sobral et al. (2013), quando avaliaram as condições higiênico-sanitárias no mercado público de Ceará, Brasil, também encontraram que o mesmo apresentava condições higiênico-sanitárias inadequadas. Estes resultados demonstram a possível inadequação das condições higiênico-sanitárias em mercados públicos que realizam a venda de alimentos em diferentes partes do mundo, ressaltando a necessidade de maior atenção nesses locais, os quais são, geralmente, muito frequentados pela população local e inclusive por turistas. É importante ressaltar que, provavelmente, grande parte dos problemas detectados nos

estabelecimentos avaliados podem ser resolvidos através da implementação de capacitações de BP aos manipuladores de alimentos e pelo provimento de condições sanitárias básicas por parte dos governos ou proprietários dos estabelecimentos.

O segundo artigo da presente Tese avaliou o conhecimento e atitudes que os manipuladores de alimentos tinham sobre segurança de alimentos, bem como a presença de *Enterobacteriaceae* em amostras de saladas de vegetais folhosos prontas para consumo e mãos de manipuladores que operavam nas ruas da cidade de Maputo, sul de Moçambique. Os resultados demostraram que a maioria dos vendedores era composta de mulheres jovens, as quais nunca tinham recebido algum tipo de treinamento em segurança de alimentos, tal como foi verificado no primeiro artigo dessa Tese.

Além disso, os resultados demonstraram que, uma grande porcentagem (42%) das manipuladoras de alimentos possuía conhecimento insuficiente sobre contaminação cruzada, controle de temperatura e patógenos alimentares. Estes resultados corraboram o que já foi relatado anteriormente em outros estudos que demostram que os vendedores de comida de rua apresentam fraco conhecimento e atitudes em segurança de alimentos em algumas partes do mundo como em Handan, na China, e em Ho Chi Minh, no Vietnam (MA et al., 2019; SAMAPUNDO et al., 2016). É de esperar o fraco conhecimento sobre contaminação cruzada, controle de temperatura e patógenos alimentares que os manipuladores de alimentos que operavam nas ruas da cidade de Maputo revelaram, pois nunca foram capacidados em assuntos de segurança de alimentos.

O mesmo problema de capacitação insuficiente dos manipuladores de alimentos de serviços de alimentação ocorreu no sul do Brasil e para contribuir com a sua resolução, o governo do Rio Grande do Sul publicou em 2009 a Portaria 78, que estabeleceu a obrigatoriedade de realização dos cursos de 16 horas, sobre BP de manipulação de alimentos aos manipuladores que trabalham em serviços de alimentação. A legislação moçambicana (Diploma Ministerial N° 51/84, de três de Outubro) não estabelece a obrigatoriedade de treinamentos em BP para os manipuladores de alimentos, o que pode contribuir com os resultados observados na presente Tese. Como sugestão, os manipuladores poderiam melhorar os seus conhecimentos, através de

participação em cursos sobre segurança de alimentos. Tais cursos poderiam ser ministrados duas vezes ao ano, por instrutores do Conselho Municipal de Maputo, que é a entidade municipal responsável, diante o pagamento de uma taxa que poderia ser acordada entre ambas as partes.

No segundo artigo também foi demonstrado que, apenas 30,80% dos 110 entrevistados responderam de forma adequada as perguntas sobre patógenos alimentares. A maioria dos manipuladores de alimentos não sabia que *Shigella* (87,3%), *Salmonella* (92,7%) e *Staphylococcus* (88,2%), eram patógenos alimentares. Resultados semelhantes a estes foram relatados por Asiegbu et al. (2016), quando estudaram o nível de conhecimento e conscientização sobre segurança de alimentos e perigos microbianos, em Joanesburgo, Africa do Sul e concluiram que mais de 70% dos vendedores de comida de rua nunca tinham ouvido falar de *Salmonella* spp. Este resultado demonstra que a excassez de conhecimentos sobre patógenos alimentares não se verifica apenas em países em desenvolvimento como é o caso concreto de Moçambique, como também em países emergentes.

Neste mesmo estudo, apenas 32,7% dos entrevistados sabiam que o aborto podia ser induzido por patógenos alimentares e apenas 10,9% dos entrevistados respondeu acertadamente que febre tifóide pode ser transmitida por alimentos. Na verdade, os problemas relacionados aos patógenos alimentares não são fáceis de serem compreendidos pelos manipuladores de alimentos que nunca foram capacitados em segurança de alimentos. A maioria dos patógenos alimentares têm nomes característicos que com muita dificuldade podem ser lembrados, principalmente se a pessoa não tiver passado por uma formação em segurança de alimentos onde tenham sido abordados esses temas.

Por outro lado, a maioria dos manipuladores (78,2%) respondeu de forma acertada as questões sobre práticas de higiene pessoal, como a necessidade de lavar as mãos antes do início da preparação dos alimentos para reduzir o risco de contaminação. Este fato induz a afirmar que os manipuladores tinham certo conhecimento sobre práticas de higiene pessoal, o que é esperado dado que questões sobre higiene pessoal são leccionados nas primeiras classes do ensino primário. Diversos estudos realizados em diferentes partes do mundo demonstraram resultados similares (AL-KANDARI; AL-ABDEEN; SIDHU,

2019b; GALAPIA-ANDOY; VALMORIDA, 2017; ISMAIL et al., 2016), ressaltando a necessidade de que segurança de alimentos seja abordada em currículos escolares infantis.

Os resultados microbiológicos das amostras das mãos dos manipuladores também revelaram que eles possuíam conhecimento suficiente sobre práticas de higiene pessoal, como a necessidade de lavar as mãos antes do início da preparação dos alimentos. *E. coli* genérica foi detectada em apenas uma amostra de mãos, indicando a correta realização desse procedimento. As contagens mais altas de *Enterobacteriaceae* observadas (saladas de folhosos prontas para consumo e mãos dos vendedores com 4,23 ± 0,78 log UFC/g e 3,14 ± 0,74 log UFC/g, respectivamente) podem ser justificadas pelo contato das mãos com vegetais naturalmente contaminados e pelo contato dos vegetais e mãos com superfícies contaminadas com esses microrgaismos. Ainda assim, tais contagens podem indicar maior cuidado com a higienização dos folhosos antes do consumo e maior cuidado com a higienização das superfícies de contato com os alimentos.

O terceiro artigo dessa Tese objetivou avaliar a prevalência de *Enterobacteriaceae* em amostras de saladas prontas para o consumo, mãos, utensílios e água utilizada para a preparação dos alimentos nos mercados da cidade de Maputo. Neste estudo vefificou-se também a presença de genes de virulência (*stx1F, stx1R, stx2F, stx2R*) em amostras que foram positivas para a *E. Coli.* Os resultados demonstraram que as contagens de *Enterobacteriaceae* variaram de 0,52 a 6,98 log UFC/g, não tendo havido uma correlação estatisticamente significativa entre as contagens bacterianas nas saladas, mãos e utensílios. No entanto, houve diferenças significativas entre os números de *Enterobacteriaceae* detectados na água e as demais amostras.

Embora, no primeiro artigo dessa Tese não tenha sido feita a avaliação microbiológica das águas utilizadas para a preparação dos alimentos, os resultados microbiológicos do terceiro artigo demontraram que água da fonte geral nos mercados não revelou presença de *Enterobacteriaceae.* Contudo, a água dos recipientes plásticos apresentou contagens médias entre 0,24 e 1,57 UFC/ml. Estas bactérias provavelmente foram provenientes dos recipientes mal higienizados.

A família *Enterobacteriaceae* é uma família heterogênea de bastonetes Gram negativos, os quais têm uma distribuição ubiquitária na natureza (SOUSA et al., 2013). A presença de níveis altos de *Enterobacteriaceae* em alimentos é indicativo da contaminação da matéria-prima, contaminação durante a preparação dos alimentos ou devido a condições inadequadas de armazenamento em temperatura elevada (ICMSF, 2006; SAHUQUILLO-ARCE et al., 2017).

Níveis insatisfatórios de *Enterobacteriaceae* (≥ 4 Log UFC/g) (ICMSF, 2006) foram detectados em 25,7% (9/35) de amostras de saladas de alface prontas para o consumo. Estes resultados são comparáveis com outros estudos realizados em alguns países africanos. Por exemplo, em Namibia, foi encontrada uma média de 4,10 log UFC/g de *Enterobacteriaceae* em alimentos vendidos nas ruas. Em Rwanda, a contagem de *Enterobacteriaceae* foi de 3,3 log UFC/g em saladas de vegetais prontas para o consumo (SHININGENI et al., 2019a; SSEMANDA et al., 2018). Essas contagens também podem indicar falta de higienização adequada das matérias-primas utilizadas para a preparação dos alimentos nestes locais.

A contaminação elevada das amostras de saladas de alface prontas para o consumo nos mercados, assim como nos ambulantes, pode ser explicada tomando como fato de que, nesses locais, não era realizada a correta higienização da matéria-prima utilizada. De acordo com a Portaria 78/2009, os vegetais frescos e folhosos, quando consumidos crus, devem ter suas folhas lavadas uma a uma com água potável, as quais, em seguida devem ser desinfetadas por imersão em solução clorada com 100 a 250ppm de cloro livre, por 15 minutos, ou com outros produtos adequados, registrados no Ministério da Saúde, liberados para esse fim e de acordo com as indicações do fabricante, sendo por fim enxaguados com água potável (RIO GRANDE DO SUL, 2009). Esta prática ainda não acontece em Moçambique de uma forma geral.

A prevalência de *E. coli* foi observada em menos de 2% das amostras, tendo sido notado a presença desta bactéria nas amostras de utensílios (bacias e facas) utilizadas durante a preparação dos alimentos. Esta contaminação dos utensílios pode ter sua origem em moscas existentes nos mercados ou devido a práticas que propiciem a contaminação cruzada. As moscas adultas podem

contaminar alimentos com microrganismos, tendo maior ou menor importância na veiculação de microrganismos patogênicos (OLIVEIRA et al., 2006).

A contaminação pode ter sua origem na água utilizada para a preparação dos alimentos reservada nos bidões. Como demonstrado no artigo um, os manipuladores de alimentos conservam a água em recipinetes plásticos, chamados de bidões, e podem contaminar a água com as suas mãos no momento em que tiram a água para a preparação dos alimentos. É de conhecimento geral, que as mãos dos manipuladores podem ser uma potencial fonte de contaminação dos utensílios e/ou dos alimentos.

Abreu et al. (2011) analisaram a qualidade microbiológica das mãos dos manipuladores que comercializavam cachorro-quente no município de Santo André e concluíram que o principal motivo pelo qual 62,5% das amostras foram positivas para a presença de coliformes, foi devido a insuficiente higiene das mãos dos manipuladores, visto que todos eles (100%) não lavavam as mãos durante o trabalho. Muitas DTA ocorrem devido à contaminação por parte dos manipuladores, durante a preparação e produção dos alimentos (TONDO; BARTZ, 2014). Portanto uma adequada higienização das mãos e utensílios é fundamental para evitar a contaminação dos alimentos (SCHUMANN et al., 2017).

Finalmente o quarto objetivo desta Tese foi avaliar a qualidade microbiana e a presença de genes de virulência de *Escherichia coli* (stx1F, stx1R, stx2F, stx2R) em duas matrizes de amostras diferentes (alface e salada de alface pronta para consumo) vendidas em cinco mercados de venda de alimentos de Maputo. Surtos de origem alimentar atribuídos à contaminação de produtos frescos em particular, vegetais de folhas verdes são uma preocupação crescente. Um total de 27 e 31 isolados foram identificados em alface e salada de alface pronta para comer em cinco mercados na Cidade de Maputo, respectivamente. *Enterobacter asburiae* foram obtidos em 26% e 13%, *Enterobacter cloacae* em 22% e 16%, *Klebsiella oxytoca* em 22% e 6%, *Escherichia hermannii* em 4% e 10% e *Escherichia coli* em 0% e 6% em alface e saladas de alface pronta para comer, respectivamente. As saladas de alface prontas para o consumo foram mais contaminadas do que a alface (matéria-prima). Nenhum gene de virulência (stx1F, stx1R, stx2F, stx2R) foi detectado pela reação em cadeia da polimerase qualitativa (qPCR) em Escherichia spp.

espécies identificadas em saladas prontas para comer. Resultados similiares foram descritos por Maistro et al. (2012) quando avaliaram a qualidade e segurança microbiológica de amostras de hortaliças comercializadas em Campinas, SP, Brasil. Entretanto, Sai & Balachandhar (2019) quando avaliaram a prevalência de *E. coli* produtora de shiga-toxina em pepino, rabanete, alface e repolho consumidos na Índia, constataram que dentre as 603 colônias de *E. coli* isoladas, a presença de genes *stx* foi confirmada em 6%. A presença de genes de virulência, como os *stx*, em microrganismos é um indicador de patogenicidade, os quais podem ser responsáveis por infecções graves para à saúde humana, como colite hemorrágica ou síndrome hemolítico-urêmica (ONLEN et al., 2017; RILEY, 2020). Embora não se tenham encontrado genes de virulência nas *E. coli* pesquisadas, não se pode afirmar que as amostras estavam livres de patógenos entéricos, como *Salmonella* e *Shigella*, os quais não foram pesquisados especificamente.

E. coli é uma espécie versátil que engloba tanto os comensais do trato digestivo de muitos vertebrados, incluindo humanos, e suas cepas patogênicas causam várias infecções intra e extra-intestinais (CLERMONT et al., 2011). À luz dos resultados do presente estudo, pôde-se observar que a *E. coli* foi isolada em poucas amostras de saladas de alface prontas para o consumo. Este resultado pode ser explicado pelo fato de que sal e vinagre foram utilizados para temperar as saladas infestigadas, e esses ingredientes podem ter inativado algumas das *E. coli* possivelmente presentes nas alfaces não higienizadas. Alguns autores consideram que o ácido acético sozinho ou combinado com sal pode inativar cepas de *E. coli* (ENTANI et al., 1998; SULAIMAN et al., 2016). Além disso, em Moçambique, os agricultores não usam com frequencia fertilizantes orgânicos provenientes de gado e de ovelhas. Estes animais são os principais reservatórios da STEC e outras cepas de *E. coli*.

Esses resultados fornecem informações valiosas que podem apoiar às decisões de segurança de alimentos, contudo, mais estudos concentrando-se em investigar patógenos e seus respectivos genes de virulência em saladas prontas para o consumo na cidade de Maputo devem ser realizados. Demais estudos também são necessários para compreender as baixas prevalências de *E. coli* em vegetais folhosos não higienizados.

Em países em desenvolvimento, como Moçambique, investigações de *E. coli* e suas cepas patogênicas são muito limitados, indicando a necessidade de examinar esse patógeno com mais detalhes. Este estudo mostra a importância de vegetais e saladas como potenciais fontes de infecção por *E. coli*.

6. CONCLUSÃO GERAL

De forma geral, a partir dos resultados obtidos na presente Tese, foi possível concluir que a contagem de *Enterobacteriaceae* variou de 0,52 a 6,98 log UFC /g e a presença de *E. coli* foi encontrada num total de 13 amostras, sendo que todas elas, foram negativas quanto à presença de genes de virulência.

A contaminação por *Enterobacteriaceae* indicou possíveis falhas nos procedimentos de higienização ou contaminação cruzada, contudo a baixa prevalência de *E. coli* e a ausência de genes de virulência nesses microrganismos indicaram que outros fatores colaboram com a baixa contaminação fecal ou com a sobrevivência desses microrganismos.

Às condições higiênico-sanitárias de preparo e venda de alimentos nos mercados públicos e nas ruas da cidade de Maputo são inadequadas, sendo, portanto, não totalmente seguro consumir alimentos nestes locais. A falta de infraestrutura básica, a falta de controle da água utilizada para preparação dos alimentos, a má conservação dos alimentos após o seu preparo e a falta de higienização dos folhosos utilizados nas saladas consumidas cruas foram alguns dos fatores que contribuiram para a inadequação dos estabelecimentos. Portanto, melhorar as infraestruturas dos locais de preparação de alimentos (mercados e ruas), assim como a realização de cursos de BP para manipuladores de alimentos e realização de posteriores inspeções periódicas são importantes para melhoria das condições higiênico-sanitárias desses locais e, consequentemnte, para a segurança dos alimentos e para a saúde pública.

Níveis baixos de conhecimentos e atitudes bem como falta de treinamento, foram observados nos manipuladores de alimentos que operavam nas ruas da cidade de Maputo. Portanto, treinamentos eficazes sobre segurança de alimentos, como ação prioritária, são necessários para melhorar os conhecimentos e, consequentemente, as práticas dos manipuladores.

A detecção de *Enterobacteriacea*e em saladas de alface prontas para consumo, água conservada em recipientes plásticos, mãos dos manipuladores e os utensílios serviu de indicativo de possíveis falhas de BP, bem como corroboram os resultados que demonstraram falhas nas condições higiênico-sanitárias. É importante que os manipuladores sejam capacitados sobre os corretos procedimentos de higienização das matéria-primas, sobretudo das alfaces, pois se sabe que pode ser um veículo importante de transmissão de alguns patógenos alimentares aos humanos.

Os vendedores de comidas prontas para o consumo que operavam nos mercados e ruas da cidade de Maputo realizavam de forma correta a lavagem de mãos, visto que *E. coli* foi encontrada em apenas uma amostra coletada de mãos de manipuladores.

Embora os resultados da presente Tese não tenham encontrado genes de virulência (*stx1F, stx1R, stx2F, stx2R*) em *E. coli* isoladas das amostras investigadas, não se pode concluir que não existem riscos de DTA associados ao alface e *E. coli* produtora de shiga-toxina, visto que as alfaces são preparadas e expostas á venda sob temperatura ambiente e sem higienização com sanitizantes. Estudos adicionais que abragem maior número de amostras são necessários para investigar a presença de *E. coli* produtoras de shiga-toxinas na cidade de Maputo, Moçambique.

Os microrganismos presentes nas amostras analisadas têm a capacidade de se multiplicar rapidamente em temperaturas de 25 e 37 º C, alcançando níveis de risco potencial. Sendo assim, mesmo que a alface apresente um baixo nível de contaminação, ela pode representar perigo ao consumidor, principalmente porque não é feita a correta higienização. Dessa forma, a implementação de boas práticas nos locais de preparo e venda de comida são importantes, assim como a conservação em um ambiente refrigerado para impedir a multiplicação dos microrganismos até quantidades que representem risco.

A partir dos resultados obtidos, constatou-se a necessidade de treinamentos e inspeções nos estabelecimentos que preparam alimentos nos mercados e ruas de Maputo, dado que deficiências nos conhecimentos e nas condições higiênico-sanitárias foram constatadas com frequência.

7. PERSPECTIVAS DO TRABALHO

Uma das perspectivas deste trabalho é de terminar o estudo sobre a prevalência de bactérias produtoras de β-lactamases em vegetais frescos vendidos nos mercados da cidade de Maputo – Moçambique. Este estudo estava sendo realizado com o objetivo de avaliar a prevalência de genes de resistência a antibióticos nos principais vegetais (alface, pepino, tomate e cebola) utilizados para a preparação de saladas prontas para o consumo em Maputo, Moçambique. Este estudo foi interrompido devido à situação atual da pandêmia de Covid-19. As análises microbiológicas desde estudo estão sendo realizado na África do Sul, em parceria com a Universidade de Pretória. Logo após a pandêmia de Covid-19 será concluída a pesquisa.

Outra pespectiva deste trabalho é capacitar os manipuladores de alimentos que operam nos mercados e ruas em Boas Práticas de manipulação, de modo que melhorem as suas práticas durante a preparação dos alimentos.

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