Brazilian Journal of Microbiology (2003) 34 (Suppl.1):123-124
ISSN 1517-8382

DETECTION OF VIRULENCE GENES IN *SALMONELLA* ENTERITIDIS ISOLATED FROM DIFFERENT SOURCES

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This paper corresponds to an "extended abstract" selected for oral presentation in the 22nd Brazilian Congress of Microbiology, held in Florianópolis, SC, Brazil, in November 17-20, 2003

ABSTRACT

The presence of three virulence genes, *inv*A, *spv*R, and *spv*C, was determined in *Salmonella* Enteritidis isolated from poultry, pigs, humans and food. All isolates were positive for the *inv*A gene, with 91.2% being positive for *spv*R and 90.2% for *spv*C. There was no significant difference in the prevalence of the virulence genes between isolates from different sources. The results indicate that there is a putative high virulence potential for the *S*. Enteritidis isolates characterized.

Key words: *Salmonella* Enteritidis, virulence, *spv*, PCR.

INTRODUCTION

Salmonellosis is one of the most common infectious diseases of both humans and animals and since the 1980's there has been a dramatic world-wide increase in the number of reported isolations of *S*. Enteritidis (6).

The virulence of *Salmonella* is linked to a combination of chromosomal and plasmid factors, the chromosomally located invasion gene *inv*A being thought to trigger the invasion of salmonellae into cultured epithelial cells (1), while an operon (*spv*RABCD), containing five genes, is present on plasmids commonly associated with some serotypes, the *spv* genes possibly having the ability to increase the severity of enteritis and allow infection and persistence at extra-intestinal sites (3).

The purpose of our study was to asses the potential virulence of *S*. Enteritidis isolates from poultry, pigs, humans and food by detecting the presence of the *inv*A, *spv*R and *spv*C virulence genes using the Polymerase Chain Reaction (PCR).

MATERIALS AND METHODS

Bacterial strains

Our study used 102 *S*. Enteritidis strains isolated in Southern Brazil, 31 from food involved in foodborne outbreaks, 22 from broiler carcasses, 21 from poultry (viscera and environmental samples), 17 from humans and 11 from pigs (lymph nodes, faeces and fresh pork sausage).

PCR

DNA was extracted as previously described (4). PCR was performed with three sets of primer pairs: 139-141, specific for the invasion gene *inv*A (4); PG 48-PG49, specific for the *spv*R gene (7); and VIR113-VIR561 specific for the *spv*C gene (5), amplifying 284 bp, 890 bp and 472 bp DNA fragments, respectively (Fig. 1). PCR amplifications were performed in a final volume of 25 µL containing DNA template, 1.5 mM $MgCl_2$, 10 mM Tris HCl (pH 8.0), 50 mM KCl, 0.2 mM of each nucleotide, 0.8 ρmol/µL of each primer and 1 U of *Taq* DNA polymerase.

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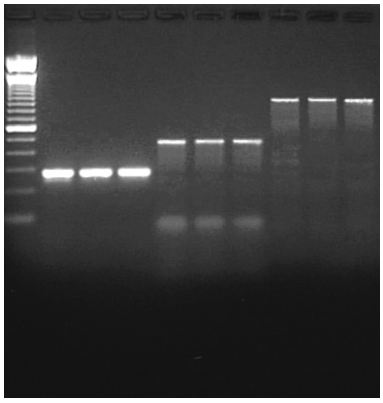

Figure 1. Electrophoreses of PCR: 100 bp molecular weight marker (lane 1); fragment from *inv*A primers (lanes 2 to 4); fragment from *spv*C primers (lanes 5 to7); fragment from *spv*R primers (lanes 8 to10).

The amplification of the *inv*A gene fragment was carried out as previously described (4), the amplification conditions for the *spv*C gene fragment being similar except that the annealing temperature was 58°C. Amplification of the *spv*R gene fragment was carried out as previously described (7). Amplification products were separated by electrophoreses on 1.2% agarose gel stained with 5 µg/mL of ethidium bromide with a 100 bp DNA ladder as molecular weight marker.

RESULTS AND DISCUSSION

All 102 *S*. Enteritidis isolates contained the invasion gene *inv*A, other studies having reported similar results (4,8), which was expected since the *inv*A is an invasion gene conserved among *Salmonella* serotypes.

The *spv*R virulence gene was detected in 91.2% of the *S*. Enteritidis strains and the *spv*C gene in 90.2%, there being no significant difference in the prevalence of virulence genes among the isolates from different sources. Only two strains contained *spv*R but not *spv*C; and one strain contained *spv*C but not *spv*R. The analysis of the *spv* operon has shown that *spv*R is an essential virulence gene while the *spv*C gene plays an accessory role but is probably needed for full virulence (2) and, therefore, it may be concluded that the isolates without the *spv*R gene are probably avirulent while absence of the *spv*C gene may indicate partial virulence.

High prevalence of these virulence genes has also been found by other authors (5,9), although in our isolates from humans and poultry the prevalence of virulence genes was even higher than that already reported. It appears that there may be some variation in the prevalence of some virulence genes because a study involving poultry products, wastewater and human sources found that only 15% of isolates contained the *spv*C gene (8).

A further conclusion of our study is that because they are not present in all isolates, *spv* genes are not appropriate targets for the specific detection of the Enteritidis serovar, although they have been used as such in several studies. However, our results regarding the presence of the *inv*A gene do agree with those of other authors who have found that this gene is a good target for detecting salmonellae.

RESUMO

Detecção de genes de virulência in *Salmonella* Enteritidis isoladas de diferentes fontes

A presença de três genes de virulência (*inv*A, *spv*R e *spv*C) foi determinada em *Salmonella* Enteritidis isoladas de aves, suínos, humanos e alimentos. Todos os isolados foram positivos para o gene *inv*A, 91,2% também foram positivos para o *spv*R e 90,2% para o *spv*C. Não existiu diferença significativa na prevalência dos genes de virulência entre isolados de diferentes origens. Os resultados indicaram que, provavelmente, exista um alto potencial de virulência nos isolados de *S*. Enteritidis caracterizados.

Palavras-chave: *Salmonella* Enteritidis, virulência, *spv*, PCR.

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