Antibiotic sensitivity profile of salmonella isolated from two slaughterhouses and human clinical cases

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Abstract

The antibiotic sensitivity of Salmonella strains isolated during the period 1996-98 from two industrial slaughterhouses of Northern Greece was determined and compared with that of salmonellae isolated from human hospital cases during the period 1995-1997. For antibiotic sensitivity the disc agar diffusion method was used. Of 1874 samples obtained from the slaughterhouse environment (floors, worker's hands and their knives), pork carcasses, by-products (livers and plucks) as well as lymph nodes and caecal contents 178 (9.5%) were positive for Salmonella spp. The salmonellae belonged to 22 serotypes. S. derby, S. london and S. typhimurium represented 25.8%, 15.2%, and 10.7% of the serotypes respectively. Of the salmonellae 59%, and 4.5%, were resistant and 33%, and 4.5% were intermediate sensitive to Tetracycin, and Streptomycin, respectively and 26.4%, 14.6%, 5.1%, 1.7% and 1% were resistant to Ampicillin, Sulfamethoxazolate / Trimethoprim, Chloramphenicol, Gentamicin, and Tobramycin respectively. Of the S. typhimurium strains 47% were resistant to Ampicillin and 41.2% to Chloramphenicol. Seven of the 19 strains were DT 104, isolated for the first time in Greece, and multiple drug resistant. Of all isolates 5.1% were resistant to Chloramphenicol, the use of which is prohibited in food animal veterinary practice. Of the 422 salmonellae isolated at the Hospital of Infectious Diseases in Thessaloniki during the period 1996-98 77.4% were S. enteritidis and 17.7% S. typhimurium. Of the salmonellae isolated during 1995-1997, 76-79% were resistant to Ampicillin and 1.2-1.5% to Chloramphenicol. Many of S. typhimurium strains isolated from the slaughterhouses and human cases exhibited the same antibiotic sensitivity profile a fact indicative of a potential transfer of animal strains to humans. Salmonellae of the same serotype exhibited different antibiotic resistance profiles, an indication of the presence of different clones within the same serotype. No S. enteritidis was isolated in slaughterhouses.

Introduction

Salmonella is a well known foodborne pathogen that is capable of producing life threatening infections. Many outbreaks as well as sporadic cases have been described worldwide annually (3,4,21). Poultry, egg, and meat products are the most commonly implicated foods in salmonella foodborne infections (9).

Since the end of the second world war antibiotics have been used in food animals because of contribution to faster growth and an increased convertibility of the feed (18). The development of antibiotic resistance is a very old issue, as old as the use of antibiotics themselves. It is apparent that the microbial antibiotic resistance is nothing more than a microbial genetic feed back to the continuing pressure generated from drug use (6). It has been a controversial issue of whether or not drug use resulted in antibiotic resistant bacterial strains which next have been transferred to humans. (12,25). An increase in the prevalence of antibiotic resistant bacteria in humans has been observed for quite some time (13). During a three year study, of salmonella strains isolated from pork and other meats it was found that their antimicrobial resistance increased from 29.1% to 50.9% (5). Almost 93% of all pigs raised in the U.S.A. (grower/finisher pigs) during the year of 1995 had been fed some form of antibiotics in their diet (7). The continuing use of antimicrobial agents in calf husbandry was implicated in the emergence of a multiple antibiotic resistant S. typhimurium (26). An antibiotic resistance study of S. typhimurium strains isolated from clinical cases and pigs indicated that 19.3% and 11.1% of the human and pig isolates respectively were resistant to at least one antibiotic. Most of the human strains were resistant to Ampicillin, Tetracyclin, and Chloramphenicol while the pig strains were often resistant to Tetracycin and Streptomycin (22).

The objectives of this study were to investigate the antibiotic sensitivity of 178 Salmonella spp. strains isolated from two industrial slaughterhouses of Northern Greece during pig processing and compare them to the sensitivity of strains isolated from human hospital cases in the same area.

Materials and Methods.

Salmonella strains.

Salmonella isolation and identification was based on
standard methodology and has been reported in the companion paper (17). All strains were serotyped at the National Salmonella Reference Center in Athens. The strains were kept lyophilized in porcelain beads in the freezer. To study the antibiotic sensitivity 1-2 beads were introduced to Tryptone Soy broth (TSB) (OXOID-Unipath Ltd, Hamshire, England), and incubated at 37°C for 24h. The next day a loop full from the TSB broth was streak onto Nutrient agar to get isolated colonies. One to five colonies were selected and transferred into 2 ml of TSB broth. The broth was incubated at 37°C till visible turbidity was observed as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) of USA (19).

Antibiotic susceptibility testing

_Salmonella_ strains were tested for their sensitivity to 12 antimicrobial agents by the disk diffusion method using Mueller-Hinton agar according to the Bauer-Kirby method (2). Using a sterile impregnated swab stick each TSB broth culture was streak onto Mueller – Hinton agar. Antibiotic discs were placed on the agar plates using the BBL antibiotic disk distribution system (Bechton Dickinson and Company automatic disk distribution system). The plates were incubated at 37°C for 18 h.

The antibiotics used included Tobramycin 10μg, Ampicillin 10μg, Amikacin 30μg (Chloramphenicol 30μg, Cefotaxime 30μg, Ciprofloxacine 5μg, Gentamycin 10μg, Tetracycline 30μg, Sulfamethoxazole-Trimethoprim 23.75-1.25μg, Ceftriaxone 30μg, Nalidix-Acid 30μg (all BBL, Cockeysville, USA) and Streptomycin 10 μg (OXOID, Unipath Ltd, Hamshire, England). The zone of growth inhibition was reported as the diameter of the zone surrounding each antibiotic disk in which bacterial growth was absent. Test strains were identified as sensitive, intermediate or resistant according to the specific BBL standards.

<table>
<thead>
<tr>
<th>Year</th>
<th>No of total salmonella isolation</th>
<th>S.enteritidis (%)</th>
<th>S.typhimurium (%)</th>
</tr>
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<tbody>
<tr>
<td>1984</td>
<td>42</td>
<td>45.2</td>
<td>14.2</td>
</tr>
<tr>
<td>1985</td>
<td>104</td>
<td>48.4</td>
<td>14.1</td>
</tr>
<tr>
<td>1986</td>
<td>45</td>
<td>40.0</td>
<td>20.0</td>
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<tr>
<td>1987</td>
<td>142</td>
<td>50.0</td>
<td>16.9</td>
</tr>
<tr>
<td>1988</td>
<td>249</td>
<td>59.0</td>
<td>26.5</td>
</tr>
<tr>
<td>1989</td>
<td>442</td>
<td>83.4</td>
<td>9.3</td>
</tr>
<tr>
<td>1990</td>
<td>429</td>
<td>84.8</td>
<td>10.3</td>
</tr>
<tr>
<td>1991</td>
<td>464</td>
<td>94.1</td>
<td>1.70</td>
</tr>
<tr>
<td>1992</td>
<td>414</td>
<td>92.2</td>
<td>7.2</td>
</tr>
<tr>
<td>1993</td>
<td>347</td>
<td>92.7</td>
<td>3.8</td>
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<tr>
<td>1994</td>
<td>487</td>
<td>93.2</td>
<td>3.7</td>
</tr>
<tr>
<td>1995</td>
<td>295</td>
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<tr>
<td>1996</td>
<td>171</td>
<td>85.3</td>
<td>11.1</td>
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<tr>
<td>1997</td>
<td>107</td>
<td>82.2</td>
<td>14.9</td>
</tr>
<tr>
<td>1998</td>
<td>144</td>
<td>64.5</td>
<td>27.7</td>
</tr>
</tbody>
</table>

Table 1. Frequency of fecal isolations of _S. enteritidis_ and _S. typhimurium_ from human gastroenteritis cases recorded by the Hospital of Infectious Diseases of Thessaloniki, Greece, during the years 1984 – 1998

collected in Greece for the years 1984-1998 by the Hospital of Infectious Diseases of Thessaloniki which serves as the Reference Center for Salmonella in Northern Greece. The data were collected from people who visited the hospital having symptoms of gastroenteritis and kept for fecal testing which later indicated the presence of _Salmonella_.

Of the twelve antibiotics used seven (Tobramycin, Ampicillin, Amikacin, Chloramphenicol, Cefotaxime, Ciprofloxacine and Gentamycin) have been chosen as the antibiotics of choice for the cure of some severe cases of human gastroenteritis and other conditions. Of the 178 salmonellae 59% and 4.5% were resistant to Tetracyclin and Streptomycin respectively. Also 33% and 4.5% of the isolates exhibited intermediate sensitivity to the same antibiotics. Furthermore 26.4%, 14.6%, 5.1%, 1.7%, and 1.1% of the isolates were found to be resistant to Ampicillin, Sulfamethoxazole/Trimethoprim (Bactrimel), Chloramphenicol, Gentamycin and Tobramycin respectively. Of the _S. typhimurium_ strains 47% were resistant to Ampicillin and 41.2% to Chloramphenicol. The percentage of salmonellae resistant to Chloramphenicol is alarming since the use of this antibiotic in food animals is prohibited. Salmonellae isolated from the slaughterhouses and

Results and Discussion

During the period from 1/7/1996 until 1/8/1998, 1874 samples were obtained from two slaughterhouses during pig processing. The samples represented the environment (floors, worker’s hands and their knives), pork carcasses, by-products (livers and plucks) as well as lymph nodes and caecal contents. A total of 178 (9.5%) were positive for _Salmonella_ spp. which belonged to 22 serotypes. _S. derby_, _S. london_ and _S. typhimurium_ represented 25.8, 15.2, and 10.7% of the serotypes respectively. Overall no _S. enteritidis_ was isolated in this study, though 10.7% of the isolated _Salmonella_ strains were _S. typhimurium_ a serotype frequently incriminated in food poisoning. It is interesting to notice that in recent years the incidence of human isolates of _S. enteritidis_ in Greece is decreasing while that of _S. typhimurium_ is increasing. Table 1 presents data.
belonging to the same serotype exhibited different antibiotic resistance profiles, an indication of the presence of different clones within the same serotype.

Amin et al., (1) found that 13 (20%) of 65 fresh pork sausages obtained from retail shops in Thessaloniki Greece harbored Salmonella of which 54% was S. typhimurium and 46% S. enteritidis. Of the 13 strains 69.2% were resistant to Ampicillin, and 7.7% were resistant to Chloramphenicol and Tetracycline (1).

A study in Germany showed that the percentage of drug resistant strains of S. typhimurium increased significantly from 27% in 1987 to 52.5% in 1996 (11).

Phage typing of our 19 S.typhimurium strains by the Danish National Veterinary Laboratory revealed that 7 (37%) belonged to DT 104 type. This is the first time this type of S.typhimurium is isolated in Greece. S.typhimurium DT 104 human isolates from many countries acquired public health significance in recent years because of their multi-drug antibiotic resistance (23, 24, 8).

Antibiotics of the 7 S. typhimurium DT 104 strains of our study revealed the same five drug resistant antibiotic profile as reported by others. The strains were resistant to Ampicillin, Chloramphenicol, Streptomycin, Tetracycline, and to Trimethoprim/Sulfamethoxazole. In Canada S. typhimurium DT 104 showed an increase of multiple drug resistance; from 2 (4.3%) of 46 isolates in 1989 to 80 (43%) of 188 isolates in 1994 (16).

Evidence from three systems used to monitor antimicrobial resistance in the USA showed that a considerable proportion (19-34%) of the S. typhimurium isolates carried markers to Ampicillin, Chloramphenicol, Streptomycin, sulphonamides and Tetracycline (10). The same authors estimated that between 68,000 and 340,000 cases of S. typhimurium infection with the five drug resistance pattern, mostly of definite phage type(DT) 104, occur in the US each year (10). The authors mentioned also that the proportions of multiple drug resistant strains have increased in the Western United States from 0.6% in 1979-80 to 19% in 1994-95.

In Canada in 1995 and 1996 37% of salmonella cultures submitted for typing to CDC exhibited multiple resistant to: Ampicillin, Chloramphenicol, Streptomycin, sulphonamides and Tetracycline. About a third of the strains exhibited also resistance to Trimethoprim or Ciprofloxacin (16).

In several European countries, S. typhimurium DT 104 has also acquired resistance to fluoroquinolones (ciprofloxacin). Fluoroquinolones are drugs of choice for treating many human infections, making the emergence of resistance to these agents of particular concern. In addition, resistant salmonella infections in humans are observed to occur as a complication of treatment for other infections in humans with an agent to which salmonellae are already resistant. This unwanted side effect of treatment converts asymptomatic transient salmonella carriers of a resistant organism into overt illness in humans (23). In England and Wales it was found that 63% (1868/2956) of the human isolates of S. typhimurium DT 104 made in 1997 were resistant to the five antibiotics and at the same time found resistant to Trimethoprim and/or Ciprofloxacin (24).

During the years 1996-1998, 422 Salmonella strains were isolated at the Hospital of Infectious Diseases in Thessaloniki. Of the isolates 77.4% were S. enteritidis and 17.7% S. typhimurium which amounts to 95.1% for both serotypes. Of the salmonella strains isolated during the period 1995-1997, 76-79 % were resistant to Ampicillin, 1.2-1.5% to Chloramphenicol, and 2.5% -7.5% were resistant to Cotrimoxazole. No resistance was recorded when the salmonella strains were tested with the following antibiotics: Cefotaxime, Amikacin, Gentamycin Tobramycin, and Ciprofloxacin. In contrast of the 178 isolates from the slaughterhouses 26.4% were resistant to Ampicillin and 5.1% to Chloramphenicol. It is interesting to notice that the antibiotic profile of S.typhimurium strains isolated from the slaughterhouses is similar to the profiles of the same Salmonella isolated from human cases at least in North America and the UK. Such similarities are indicative of a potential transfer of antibiotic resistant animal salmonella strains to humans.

According to one study most of the human S. enteritidis (98%) isolates in Greece were found to be Ampicillin resistant (14). Of the 491 Salmonella spp strains isolated from patients with diarrhoea in the island of Crete during the 3-year period of 1992-1994 (19), 40.7%, 36%, 4.7%, 3.5%, 1.6%, 0.4% and 0.2 % were resistant to Tetracycline, Ampicillin, Chloramphenicol, Trimethoprim/Sulfamethoxazole, Neomycin, Gentamycin and Norfloroxacin respectively. S. enteritidis, S. typhimurium, S. virchow and S. newport constituted 82.7%, 11.2%, 1.3% and 1% of the strains.

There are some trends in antibiotic resistance. One of the most known trends is the observation that organisms resistant to one antibiotic are more likely to become resistant to others and also that resistance, once acquired, declines slowly, if at all (15). Thus in both animal husbandry and medical practice it is vital that the wider implications of antibiotic use are considered before writing prescriptions.
References


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