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*Research Article*

# Antibiotic Resistance of *Salmonella* Isolated from Muscat, Oman

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## Abstract

One hundred and fifty-nine different strains of *Salmonella* species from human and non-human materials were isolated. The isolation was carried out according to standard methods and the identification was done by API 20E system. The isolates were typed to serological O groups using a slide agglutination test by polyvalent O antisera. All strains were exposed to eighteen different antibiotics following the disk diffusion method. Of the 159 strains, 48 showed resistance to one or more antibiotics. Strains isolated from chickens shared resistance to kanamycin, neomycin and trimethoprim. The strains from sheep shared resistance to tetracycline and the strains from humans shared resistance to trimethoprim. None of the strains were resistant to cyclosporins and amikacin. Various strains were also found to be resistant to other antibiotics.

## Introduction

Salmonellosis is a zoonotic disease which occurs as enteric fever, gastroenteritis, empyema, bone and joint infections, or a combination of these complications (Hadfield, 1985). The widespread use of various antibiotics for prevention and treatment of bacterial infections, including salmonellosis, in man and animals, and the use of antibiotics as growth promoters has selected for the emergence of antibiotic resistant strains. The antibiotic resistant pathogens can be ingested via food and water and cause major outbreaks (Holmberg *et al.*, 1984).

Unlike the situation in developed nations, laboratory analysis of salmonellosis in most other countries, including Oman, continues to be incomplete (Young, 1986). In Oman, there has been no major effort to identify the strains of existing *Salmonella*. Therefore, little is known about their drug-susceptibility. In this investigation, attempts are made to screen the drug susceptibility of *Salmonella* strains isolated from human and non-human sources in the Muscat region, Sultanate of Oman

## Materials and Methods

Different *Salmonella* strains were isolated from human and non-human sources in the region of Muscat, Sultanate of Oman. The human *Salmonella* strains were obtained from pus in the breast, urine, stool, and blood. Additional strains were isolated from food handlers and stool samples. The non-human strains were obtained from chicken and sheep intestines and raw sewage. The isolation and identification procedures were carried out according to the standard methods of isolation (Sonnewirth, 1980). The identification was done by API 20 E System (API Analytical Products, New York, NY). The isolated strains were then typed to serological O group using slide agglutination test by polyvalent O antisera (Kaufmann, 1972). After identification and grouping, cultures were grown on peptone agar slants for storage as recommended by Minor (1984). The cultures were then actively grown and screened against eighteen antibiotics (Table 2) following the method of Bauer *et al.* (1966).

## Results

One hundred and fifty-nine different strains of *Salmonella* belonging to different O serological groups were isolated (Table 1). Of these, 42 strains were isolated from non-human sources and 117 from human sources. A majority of the strains (64 strains) were identified as group B, 33 as C, 29 as E, 21 as D and the remaining 12 strains to various other groups. A significant number of the strains (30 strains) in group B were isolated from chicken. Also, most of the non-human strains (19.5%) were isolated from chickens, whereas most strains from humans were from stool (62.2%).

Eighteen drugs were employed for screening the strains for their antibiotic susceptibility (Table 2). The strains were considered either sensitive or resistant according to the diameter of zone sizes that correspond to the control *E. coli* standard. Forty-eight (30.1%) strains were resistant to at least two drugs were later numbered sequentially as strains 1 through 48. The highest frequency of resistance was with tetracycline (26.4%). Only one strain from group B was resistant to gentamicin (0.6%). None of the resistant strains exhibited resistance to amikacin, tobramycin, and to any of the four cephalosporins. A higher frequency of drug resistance was detected with group B. In contrast, no resistant strain was detected from E, G, I, M, and O groups. Table 3 shows the antibiotic resistant pattern and the source of each strain. The broadest range of drug resistance to 10 different antibiotics was shown by strain 40 of group B, isolated from a 30-year old patient suffering from diarrhea. Many of the strains isolated from chicken were also resistant to eight different antibiotics. These strains isolated from chickens shared resistance to three antibiotics, kanamycin, neomycin, and tetracycline. For convenience, this antibiotic resistance pattern was labeled as Group I. The Group II was resistant to an additional antibiotic - minocycline. Additional resistance to other antibiotics was recorded in Group III isolates. The Group IV strains, which were isolated from sheep, had antibiotic pattern of kanamycin, minocycline and tetracycline. All other resistant strains showed a variety of resistance

Table 1: Sources of *Salmonella* strains

| Salmonella O Group | NO. (%) of Isolates | Non-Human Sources | | | Human Sources | | | | | | Stool | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Chicken | Sheep | Sewage | Blood | CSF* | Wound | FP* Vomit | Pus from breast | Urine | IP* | FP* | FH* |
| A | 2(1.2) | 1(0.6) | - | - | 1(0.6) | - | - | - | - | - | - | - | - |
| B | 64 (40) | 30(18.2) | - | . | 1(0.6) | - | - | - | 1(0.6) | - | 9(5.6) | 2(1.2) | 12(7.6) |
| C | 33(20.7) | - | 9(5.6) | - | - | - | 1(0.6) | - | - | 2(1.2) | 8(5) | - | 1(0.6) |
| D | 21(13.2) | - | - | - | 9(5.6) | - | - | 1(0.6) | - | - | 8(5) | 6(3.7) | 13(8.1) |
| E | 29(18.2) | - | - | 1(0.6) | - | 1(0.6) | - | - | - | - | 8(5) | - | - |
| G | 5(3.1) | - | - | - | 1(0.6) | - | - | - | - | - | 3(1.9) | - | - |
| I | 2(1.2) | - | - | - | - | - | - | - | - | - | 1(0.6) | - | - |
| M | 1(0.6) | - | - | - | - | - | 1 | 1 | 1 | - | 2(1.2) | - | - |
| O | 2(1.2) | - | - | - | - | 1 | - | - | - | 2 | - | - | - |
| Total | 159 | 31 | 9 | 1 | 12 | 1 | 1 | 1 | 1 | 2 | 60 | 10 | 29 |
| Total % | | (19.5) | (5.6) | (0.6) | (7.5) | (0.6) | (0.6) | (0.6) | (0.6) | (1.2) | (37.7) | (6.3) | (18.2) |

*IP = Inpatients; FP = Food Poisoning, FH = Food Handlers (people who worked with food materials; CSF = Cerebro Spinal Fluid.)

Table 2: Antibiotic susceptibility of 159 Salmonella strains

| Salmonella O group | No of isolates | No* (%) of resistant | % of Salmonella groups resistant to individual drug | | | | | | | | | | | | | | | | | |
| | | | Amino glycosides* | | | | | | Cephalosporins* | | | | C* | Na* | Penicillins* | | Solfonamides* | | Tetracyclines* | |
| | | | An | Gm | N | K | S | Tm | Cr | Cro | Fox | Ma | | | Am | Cb | Smx | Tmp | Min | Te |
| A | 2 | 1(50) | - | - | 50 | 50 | - | - | - | - | - | - | - | 50 | - | - | - | 50 | 50 | 50 |
| B | 64 | 38(59.3) | - | 1.6 | 47 | 48.1 | 26.1 | - | - | - | - | - | 6.2 | 23.4 | 9.3 | 8 | 34.3 | 34.3 | 42.2 | 54.7 |
| C | 33 | 7(21.2) | - | | | | | | | | | | 9 | - | | | 9 | 6 | 18.1 | 21.2 |
| D | 21 | 2(9.5) | - | | | | | | | | | | | | | | 9.5 | 4.7 | | |
| E | 29 | - | | | | | | | | | | | | | | | | | | |
| G | 5 | - | | | | | | | | | | | | | | | | | | |
| I | 2 | - | | | | | | | | | | | | | | | | | | |
| M | 1 | - | | | | | | | | | | | | | | | | | | |
| O | 2 | - | | | | | | | | | | | | | | | | | | |
| Total No | 159 | 48 | - | 1 | 34 | 37 | 19 | - | - | - | - | - | 7 | 17 | 6 | 5 | 27 | 26 | 32 | 42 |
| Total % | | 30.1 | - | 0.6 | 21.3 | 23.2 | 11.9 | - | - | - | - | - | 4.4 | 10.7 | 3.8 | 3.1 | 16.9 | 16.3 | 20.1 | 26.4 |

*An-amikacin; Gm-gentamicin; n-neomycin; K-kanamycin; S-streptomycin; Tm-tobramycin; Cr-cephalothin; Cro-ceftriaxone; Fox-cefoxitine; Ma-cephamandole; Chloramphenicol; Na-nalidixic acid; Am-ampicillin; Cb-carbenicillin; Smx-sulphamethoxazole; Tmp-trimethoprim; Min-minocycline; Te-tetracycline; No-Number.

Table 3. Antibiotic resistance pattern of *Salmonella* strains

| No. | Serotype | Resistance Pattern | No. of drugs | Ab Resistance Grouping | Source |
|---|---|---|---|---|---|
| 1 | B | KNTe | 3 | I | Chicken |
| 2 | B | KMinNTe | 4 | II | Chicken |
| 3 | B | KMinNTe | 4 | II | Chicken |
| 4 | B | KMinNTe | 4 | II | Chicken |
| 5 | B | KNTe | 3 | I | Chicken |
| 6 | B | KMinNNaTe | 5 | * | Chicken |
| 7 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 8 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 9 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 10 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 11 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 12 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 13 | B | KMinNNaSSmxTeTmp | 8 | * | Chicken |
| 14 | A | MinNaTeTmp | 4 | * | Chicken |
| 15 | B | KMinNNaTeTmp | 6 | III | Chicken |
| 16 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 17 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 18 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 19 | B | KMinNNaSSmxTeTmp | 8 | III | Chicken |
| 20 | B | KMinNNaSSmxTeTmp | 8 | I | Chicken |
| 21 | B | KNTe | 3 | II | Chicken |
| 22 | B | KminNTe | 4 | II | Chicken |
| 23 | B | KminNTe | 4 | I | Chicken |
| 24 | B | KNTe | 3 | I | Chicken |
| 25 | B | KNTe | 3 | II | Chicken |
| 26 | B | KMinNTe | 4 | II | Chicken |
| 28 | B | KMinNTe | 4 | * | Chicken |
| 29 | B | CKMinNSSmxTeTmp | 7 | * | Chicken |
| 30 | B | CKMinNSSmxTeTmp | 7 | III | Chicken |
| 31 | B | KMinNNaSSmxTeTmp | 8 | IV | Sheep |
| 32 | C | KminTe | 3 | IV | Sheep |
| 33 | C | KMinTe | 3 | * | Sheep |
| 34 | C | CSmxTe | 3 | IV | Sheep |
| 35 | C | KMinTe | 3 | IV | Sheep |
| 36 | C | KMinTe | 3 | | Sheep |

Human Strains

| No. | Serotype | Resistance Pattern | No. of drugs | Ab Resistance Grouping | Source |
|---|---|---|---|---|---|
| 37 | D | SMxTmp | 2 | * | Blood (48 yr. old male patient with septicemia) |
| 38 | C | CMinSmxTeTmp | 5 | * | Stool (Male patient with gastroenteritis) |
| 39 | D | Ssmx | 2 | * | Stool (6 yr. old male patient with gastroenteritis) |
| 40 | B | AmCCbGmKMinSSmxTeTmp | 10 | * | Stool (30 yr. old male patient with diarrheal |
| 41 | B | AmCbSmxTeTmp | 5 | * | Stool (19 yr. old female patient with food poisoning) |
| 42 | B | MinTe | 2 | * | Stool (2 yr. old female patient with gastroenteritis) |
| 43 | C | CKMinNSSmxTeTmp | 8 | * | Stool (9 mo. male patient with gastroenteritis) |
| 44 | B | AmCbSSmxTmp | 5 | * | Stool (6 yr. old female patient with gastroenteritis) |
| 45 | B | CKNSmx | 4 | * | Stool (4 yr. old female patient with gastroenteritis) |
| 46 | B | AmCCbSSmxTeTmp | 7 | * | Stool (10 mo. old male patient with diarrhe |
| 47 | B | AmSmxTeTmp | 4 | * | Stool (1 yr. old female patient with diarrhe |
| 48 | B | AmCbSSmxTmp | 8 | * | Stool (1 yr. old male patient with diarrhe |

* Antibiotic resistant of ungrouped strains

Table 4: Effect of long storage on drug resistance of Salmonella strains.

| Strian | At the time of isolation* | | 12 month of storage | | | |
|---|---|---|---|---|---|---|
| | Resistance Pattern | No. of Drug | Resistance Pattern | No. of Drug | Resistance to drug lost | Drug lost |
| 9 | KMinNNaSSmxTeTmp | 8 | MinNNaSSmxTeTmp | 7 | K | 1 |
| 30 | KMinNSSmxTeTmp | 7 | KMinNSS | 6 | Tmp | 1 |
| 33 | KminTe | 3 | KTe | 2 | Min | 1 |
| 35 | KminTe | 3 | - | 0 | KMinTe | 3 |
| 39 | Ssmx | 2 | Smx | 1 | S | 1 |
| 43 | CKMinNSSmxTeTmp | 8 | CKMinNSmxTeTmp | 7 | S | 1 |

patterns to different antibiotics and they were left ungrouped. The strain number 6, 14, 15, 29 and 30 shared common resistance to neomycin and tetracycline. Of the 2 human strains that were resistant to different antibiotics, 9 strains shared resistance to trimethoprim, 8 strains shared resistance to sulfamethoxazole, and 7 strains shared resistance to tetracycline.

The storage of the cultures for longer period has caused six of the strains to lose their resistance to some antibiotics (Table 4). It was observed that the strain 35 which was resistant to three antibiotics after six months of storage has lost its resistance entirely after 12 months of storage.

## Discussion

One hundred and fiftynine (159) different strains of Salmonella screened in the present study, belong to different O serological groups, i.e., 64 were from Group B, 3 from Group A, 29 from Group E, 21 from Group D and 2 from A, G, I, M and O groups. Group B strains were collected from stool (62.2%) and chicken (18.2%). Since most of the Group B strains (80.4%) were from chicken and human, it is possible that chicken salmonellae were ingested via food by human and caused various diseases such as food poisoning and were subsequently isolated from human stools.

Although 111(70%) strains did not show any antibiotic resistance, 48 strains (30%) were resistant to at least two of the antibiotics tested. About 18 (50%) isolated from chicken and human showed resistance to 7-10 antibiotics. The number of resistant organisms were 38 (59.3%) in Group B, 7(21.2%) in Group C, 2(9.5%) in Group D, and (50%) in Group A. In general, Salmonella strains isolated from chickens showed a common antibiotic resistance pattern of KNTe. Additionally, many of these salmonellae also showed resistance pattern of MinNaTmp. Interestingly, Salmonella strains that were isolated from sheep gave resistance pattern of KMinTe which is very similar to the resistance pattern of many Salmonella strains isolated from chickens. This raises the possibility of cross infection between sheep and chicken and of common origin of the resistant strains.

Most of the Salmonella strains from humans shared a resistance pattern of AmTmp, and did not bear much similarities to sheep or chicken strains. The spread of S. phimurium and other salmonellae from animal to human

is well documented, and yet the available data still remain controversial (Holmberg et al., 1984). There are also other reports of great similarities in the resistant patterns and phage types of animal and human salmonellae (Neu et al., 1975). Although no clear similarities were found in this study between antibiotic resistance pattern between animal and human Salmonella isolates, it does not exclude the possibility that resistant strains from animals could not be transferred to humans. Both pathogenic and non-pathogenic strains resistant to drugs may be transported from animals to humans via food (Linton et al., 1977). Undoubtedly, the improper and unnecessary use of antimicrobial drugs in humans also promote development of resistant strains.

For decades chloramphenicol has been the drug of choice in treating S. typhi (Spika et al., 1987). Some countries have reported that chloramphenicol resistant salmonellae have increased drastically, whereas in other countries, chloramphenicol resistant salmonellae have not yet been isolated (Hadfield et al., 1985, and Murray et al., 1985). Seven of our strains (strain 29, 34, 38, 40, 43, 45, and 46), of Group B and C, showed resistance to chloramphenicol. To our knowledge, this is the first report of chloramphenicol-resistant salmonellae isolated from Oman. The chloramphenicol resistance in S. typhi might have resulted due to its improper use and high endemicity of S. typhi (Yohannes 1985).

There are reports that S. typhi and other salmonellae have unstable antibiotic resistant plasmids which may be lost in prolonged storage (Murray et al., 1985). In the present investigation five strains (strain 9, 30, 33, 39 and 40) had lost their resistance to at least one antibiotic during prolonged storage. The strain 35 had completely lost its resistance to four different antibiotics.

Almost all animal strains showed antibiotic resistance to KNTmp. On the other hand, the strains from humans shared resistance only to trimethoprim. The human and animal isolates did not exhibit a common antibiotic resistant pattern which is probably due to unequal distribution of O serogroups with regard to their source of isolation. The dissimilarity in resistance pattern between human and non-human isolates does not disapprove antibiotic resistant bacteria in animals are not transferred to humans. The common antibiotic resistance pattern from animals to human strains may be demonstrated during outbreaks of antibiotic resistant salmonellae. In such outbreaks,

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immediate action must be carried out to isolate resistant strains in order to analyze their antibiotic pattern. Any delay in analyzing strains that are responsible for outbreaks can obscure the actual source of an outbreak. This is because many resistant strains tend to lose their resistance to antibiotics after longer storage periods and maintenance of cultures, or due to genetic changes during cell division or transmission from one host to another as observed in some strains.

In both animals and human strains, the highest frequency of resistance to drugs was observed with tetracycline. Since tetracycline is one of the broad spectrum antibiotic, improper use of such drugs for prophylaxis and for growth promotion has led to the development of multiple resistant bacteria.

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## References

Bauer, A., W. Kirby, W. Sherris and M. Turk, 1966. Antibiotic susceptibility testing by standard single disk method. Am. J. Clin. Pathol., 45:493-96.

Hadfield, T., M. Monson and I. Wachsmuth, 1985. An outbreak of antibiotic resistant Salmonella entendis in Liberia, West Africa. J. Infect. Dis., 151:790-95.

Holmberg, S., M. Osterholm, A. Kenneth, K. Senger and M. Cohen, 1984. Drugresistant Salmonella from animals fed antimicrobials. N. Engl. J. Med., 311: 617-22.

Kaufmann, F., 1972. Determination of antigens. Muskgard. Copenhagen.

Linton, A., K. Howe, P. Bennet, M. Richmond and E. Whiteside, 1977. The colonization of the human gut by antibiotic resistant E. coli from chickens. J. Appl. Bacteriol., 43:465-9.

Minor L., 1984. Genus Salmonella, p. 427-58. In N. Krieg, and J. Holt (eds.), Bergey's manual of systemic bacteriology. Vol. 1. Williams and Wilkins. Baltimore.

Murray, B., N. Levine, A. Cordano, K D'Ottone, P. Jananetra Kopecko, R PanUrae and I. Prenzel, 1985. Survey of plasmids in Salmonella typhi from Chile and Thailand. J. Infect. Dis., 151: 551-55.

Neu, H., C. Cherubin, E. Longo, B. Flouton and J. Winter. 1975. Antimicrobial resistance and R-factor transfer among isolates of Salmonella in the Northeastern of the United States: A comparison of human and animal isolates. J. Infect. Dis., 132-617-22.

Ralph, A., 1986. In Salmonella, p. 452-58. In S. Baron, (ed.), Medical microbiology. 2nd Edition. Addison-Wesley. California.

Sonnenwirth, A. 1980. Gram-negative bacilli, vibrios, and spirilla, p. 1731-852. In A. Sonnenwirth, and L. Jaret. Gradwohl's clinical laboratory methods and diagnosis. Vol. 1. 8th Edition. C. V. Mosby Company. St. Louis.

Spika, J., S. Waterman, G. Soo Hoo, M. St. Louis R. Pager, S. James, M. Bissett, L. Mayer, J. Chiu, B. Hall, K. Green, M. Potter, M. Cohen and Blake, 1987. Chloramphenicol-resistant Salmonella newport traced through hamburger to diary farms: a major persisting source of human salmonellosis in California. N. Engl. J. Med., 316:565-70.

Yohannes, A. 1985. Salmonella from Ethiopia: Prevalent species and their susceptibility to drugs. Ethiop. Med. J., 23:97-102.

Young, L., 1986. Antimicrobial resistance implications for antimicrobial use. Am. J. Med. 80 (Suppl. C):35-9.