Quinolone resistance in non-typhoidal *Salmonellae* isolated from patients having primary extra-intestinal infections

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Global emergence of multiple drug resistance in non-typhoidal *Salmonellae* is a serious public health concern and is posing a challenge in the management of intestinal and extra intestinal infections due to these organisms. After appearance of resistance to ampicillin, chloramphenicol and trimethoprim and sulphamethoxazole, which are the first line antimicrobial agents; the quinolones due to their proven efficacy, remain the treatment of choice for non-typhoidal *Salmonellae* intestinal and extra-intestinal infections. Lately, appearance of resistance to quinolones in non-typhoidal *Salmonellae* is a disquieting signal for the health authorities.[1,2] Nalidixic acid resistance has been associated with reduced clinical response and treatment failure to ciprofloxacin therapy and this type of resistance is being lately observed in many countries. Ciprofloxacin resistance in non-typhoidal *Salmonellae* is being encountered more frequently in Europe.[1]

The present study describes the prevalence of extra-intestinal non-typhoidal *Salmonellae* and its resistance to quinolones (nalidixic acid and ciprofloxacin) among the strains isolated from the Al-Hasa region of the Kingdom of Saudi Arabia (KSA).

The study was carried out at the 500-bed King Fahad Hospital and Tertiary Care Center, Al-Hofuf, Al-Hasa, KSA. The non-typhoidal *Salmonellae* were isolated from various extra-intestinal clinical samples received from the patients admitted in the hospital during the period of 1999 - 2002. Pus samples and wound swabs were cultured on blood agar and MacConkey's agar. Cerebrospinal fluid (CSF) and other body fluid samples were directly cultured on blood agar, MacConkey's agar, chocolate agar and were also inoculated in brain heart infusion broth, which was sub cultured on the above mentioned media after 24 hours of incubation. Blood culture samples were collected in the Bactec 9240 blood culture bottles and processed in Bactec 9240 analyzer (Becton Dickinson Co, Maryland, United States of America). The positive blood culture bottles were sub cultured on blood agar, MacConkey's agar and chocolate agar. Urine samples were cultured on blood agar and cystine lactose electrolyte deficient medium. Fecal samples were collected from the patients from whom non-typhoidal *Salmonellae* were isolated from extra-intestinal sites to confirm the source of infection, and the patients in whom the non-typhoidal *Salmonellae* were also isolated from the fecal samples, were excluded from the study. In case of repeated isolation of *Salmonellae* from the same patient only the first isolation was counted. Salmonella strains were identified by API20E system (bioMerieux Vitek, SA, France) and serogrouped using somatic group Salmonella A-G antiserum (Murex Biotech Ltd, United Kingdom (UK)). Antibiotic susceptibility was performed by disc diffusion technique according to the criteria of the National Committee for Clinical Laboratory Standards. Minimum inhibitory concentration (MIC) for nalidixic acid and ciprofloxacin was determined by agar dilution method.

During the 4 years of study, 64 strains of non-typhoidal *Salmonellae* were isolated from blood, wound swabs, pus, urine and body fluid samples. Salmonella strains belonging to serogroups B, 19 (29.6%) and D, 20 (31.2%) were common followed by serogroup C1, 12 (18.7%). Salmonella strains belonging to serogroups C2, 4 (6.3%) and E, 3 (4.6%) were the least encountered. The majority of the strains were isolated from blood 27 (42.1%) followed by 20 (31.2%) from wound swabs and 8 (12.5%) from the pus samples. While 4 (6.2%) of the strains were isolated from synovial fluid, 2 (3.1%) from CSF and 2 (3.1%) from urine. Multiple drug resistance (resistance to 2 or more than 2 antibiotics) was observed in 8 (12.5%) of the strains. Resistance to 6 antimicrobials agents (ampicillin, amoxicillin and clavulanic acid, chloramphenicol, trimethoprim and sulphamethoxazole, nalidixic acid, ciprofloxacin) was observed in 2 (3.1%), 4 antimicrobial agents (ampicillin, amoxicillin and clavulanic acid, chloramphenicol, nalidixic acid) in 4 (6.3%) and 2 antimicrobial agents (ampicillin, trimethoprim and sulphamethoxazole) in 2 (3.1%) of the strains. While 9 (14.0%) strains had resistance to only one antimicrobial agent (nalidixic acid 8, trimethoprim and sulphamethoxazole 1). All the isolated strains were sensitive to cephalothin, cefoxitin, cefotaxime, ceftriaxone, gentamicin, amikacin, aztreonam, imipenem and piperacillin. Isolated resistance to nalidixic acid was more commonly observed 8/64 (12.5%). Resistance to nalidixic acid was more common among strains isolated from blood 5 (35.7%), followed by the strains isolated from a wound swab 3 (21.4%). Nalidixic acid resistance was higher among the strains belonging to serogroup C1, 8 (57.1%), followed by serogroups D, 5 (35.7%) and G, 1 (7.1%). Out of 8 nalidixic acid resistant strains of serogroup C1, 2 were also resistant to ciprofloxacin. None of the strains

Table 1 - Nalidixic acid resistance in non-typhoidal *Salmonellae* during 1999 - 2002.

Source	Strains isolated	Salmonella serogroups nalidixic acid resistant						Total
	n	B	C1	C2	D	E	G	(%)
Blood	27	-	3	-	2	-	-	**5 (35.7)**
Wound	20	-	2	-	1	-	-	**3 (21.4)**
Abscess	8	-	-	-	1	-	-	**1 (7.1)**
Synovial fluid	4	-	1	-	1	-	-	**2 (14.2)**
CSF	2	-	1	-	-	-	-	**1 (7.1)**
Urine	2	-	1	-	-	-	1	**2 (14.2)**
Pleural fluid	1	-	-	-	-	-	-	**- -**
Total	**64**	**-**	**8 (57.1)**	**-**	**5 (35.7)**	**-**	**1 (7.1)**	**14**

CSF - cerebrospinal fluid

belonging to serogroups B, C2 and E had nalidixic acid resistance, while one strain of serogroup G was resistant to nalidixic acid (**Table 1**). An increasing trend in nalidixic acid resistance was observed during the 4 years of study period. This increased from 0/11, 0% in 1999, 1/11, 9% in 2000, 2/19, 10.5% in 2001 and 11/23, 47.8% in 2002. There was 11 times increase in the nalidixic acid resistance during the study period. Minimum inhibitory concentration to nalidixic acid and ciprofloxacin of the non-typhoidal *Salmonellae* isolated from extra-intestinal sources was carried out in 11 nalidixic acid resistant strains isolated during 2002. Minimum inhibitory concentration to nalidixic acid was >256 μg/ml in all the 11 strains and MIC to ciprofloxacin of the 2 ciprofloxacin resistant strains was 8 μg/ml. Out of 11 nalidixic acid resistant strains, 3 (4.6%) strains had reduced susceptibility to ciprofloxacin (MIC 0.20 μg/ml).

Emergence of resistance to quinolones is a disquieting quandary as the resistance to this drug is chromosomal in nature due to a point mutation leading to an amino acid change of gyrase subunit A gene (gyrA) and gyrase subunit B gene (gyrB) unlike in other antibiotics, where it is plasmid mediated which suggests that this resistance has emerged as a result of selective pressure of the excessive use of quinolones.[3,4] Resistance to nalidixic acid among human isolates of non-typhoidal *Salmonellae* in Denmark increased from 0.8% in 1999 to 8.5% in 2000 and in Finland it increased from 3.9% in 1995 to 23.5% in 1999. This rise in resistance was more among the strains isolated from the patients who had recent history of travel to the South Eastern Asian Countries.[5] According to the year 2000 report of the international surveillance network for the enteric infections - Salmonella and VTEC O157 (Enter-net) from 10 European countries, 14% of the human non-typhoidal *Salmonellae* is resistant to nalidixic acid and 0.5% to ciprofloxacin.[1] In the present study, the overall resistance to nalidixic acid among non-typhoidal *Salmonellae* associated with extra-intestinal infections was higher (21.8%) than that reported from the European countries and this resistance increased from 0% in 1999 to 47.8% in 2002. Resistance to ciprofloxacin was observed in 2 (3.1%) of the strains and this resistance appeared suddenly in 2002. Resistance to nalidixic acid was observed more frequently among the strains belonging to serogroups C1 and D. Studies from the UK reports that the Salmonella Virchow (serogroup C1) strains resistant to nalidixic acid having enhanced invasive potential are more commonly associated with septicemia.[1] In the present study 3/5 nalidixic acid resistant strains of *Salmonellae* isolated from blood, belonged to serogroups C1 and overall Salmonella serogroups B (29.6%) and D (31.2%) were more commonly associated with the extra-intestinal infections. The present study suggests that the non-typhoidal *Salmonellae* are capable of causing primary extra-intestinal infections and this possibility should be kept in mind while treating the patient with empirical antibiotic therapy. This also indicates that apart from the host factors there may also be some difference in the strains causing intestinal and extra-intestinal infections. Serogroups B, C1 and D were more commonly associated with septicemia, while serogroup D was predominantly involved in

pyogenic abscesses. However, further observation with a large sample size is required to substantiate these findings.

Emergence of quinolones resistance in non-typhoidal *Salmonellae* has limited the choice of oral drugs for treatment of extra-intestinal infections. The mutational resistance to quinolones is promoted under selective pressure of these drugs, being used frequently in humans and animals. Enrofloxacin a fluoroquinolone used in animals can select out the Salmonella mutants, which are resistant to nalidixic acid and ciprofloxacin. Nalidixic acid has been extensively used in the developing countries for the treatment of bacillary dysentery, which led to the emergence of nalidixic acid resistance in *Shigella dysenteriae*.[1] Extensive use of nalidixic acid for the treatment of urinary tract infection and other infections can lead to the development of resistance to this drug among the normal coliform gut flora. All these factors could be together generating the selective pressure for emergence of quinolone resistance in non-typhoidal *Salmonellae*.

The increasing resistance to quinolones in non-typhoidal *Salmonellae* causing extra-intestinal infections calls for reduction in the selective pressure by restriction in use of these drugs and continuous surveillance on quinolones resistance in *Salmonellae*.

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Hemodialysis and ultrafiltration. *A bridge to cardiac surgery*

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Hemodialysis (HD), hemofiltration (HF) and ultrafiltration (UF) are being extended to non-renal applications such as end stage heart failure awaiting cardiac transplantation, liver failure, and drug over dose. We report on the increase of need for UF and HD in a chronic renal failure patient with severe acute heart failure following acute aortic regurgitation due to infective endocarditis. Here, we discuss the widening of the application of UF and HD related treatment in other specialties.

A 34-year-old white male was admitted to the hospital with a 2-day history of shortness of breath and cough productive of whitish sputum. Background history revealed that he was involved in a motor vehicle accident 10 years earlier causing damage to renal arteries and loss of kidneys, resulting in end stage renal failure. He was enrolled into the chronic hemodialysis program and was stable for 10 years on HD, via left radial arteriovenous fistula (3 times per week; 4 hours per session). On examination, he was pale, pyrexial and distressed. His blood pressure was 100/53 mm Hg and jugular venous pressure was elevated. He had 2/6-ejection systolic murmur at left sternal border and bi-basal crepitations. He was treated with intravenous cefuroxime, UF and HD. Five days later, a new murmur, 3/4 early diastolic murmur was detected at the lower left sternal border, associated with hypotension. There were no clinical stigmata of infective endocarditis. Echocardiogram showed vegetations on aortic valve with significant aortic regurgitation. One out of 3 blood cultures grew S*taphylococcus aureus*. He was put on intravenous vancomycin. However, he did not improve and subsequently he underwent successful aortic valve replacement surgery. While awaiting surgery, he was on alternate days UF and HD initially, there after he required daily dialysis support for his severe cardiac failure. Postoperatively, he was put back on his usual HD regimen of 3 times per week and remains well.