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Fluoroquinolone Resistance in Salmonella: A One Year Study of Blood Cultures at Sheikh Zayed Hospital, Lahore

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ABSTRACT

Objective: To study the proportion of fluoroquinolone resistance in *Salmonella* species isolated from blood cultures. **Study Design:** Retrospective Study. **Place and duration of study**: Department of Microbiology, Sheikh Zayed Hospital Lahore from 1st June 2014 to 30 May 2015. **Materials and Methods**: All Salmonellas isolated from blood culture were included in this study and their antimicrobial susceptibility patterns analysed for fluoroquinolone resistance using the new CLSI 2014 guidelines. **Results**: A total of 5273 blood cultures were received in the laboratory during the study period. *Salmonella* was isolated from 41 cultures, out of which 35 were *Salmonella typhi* (85.4%) and 6 (14.6%) were *Salmonella paratyphi-A*. Fluoroquinolone resistance was seen in 34 (82.9%) and susceptibility in 7 (17.1%) isolates. All these 41 isolates were sensitive to ceftriaxone, cefotaxime, cefixime, imipenem/meropenem and aztreonam. Chloramphenicol when available also showed a susceptibility of 100 %. Ampicillin was resistant in 32/41 (78 %) strains. **Conclusion**: Quinolone resistance is on the rise in *Salmonella* species. Newer criteria published by the CLSI in 2014 for interpreting fluoroquinolone susceptibility in *Salmonella* must be implemented, and antibiotic policies must be developed and strictly enforced.

Key words: *Salmonella*. Blood culture. Fluoroquinolones. Antimicrobial resistance.

INTRODUCTION

The "enteric fevers" (typhoid fever) caused by *Salmonella typhi* remain a major public health problem in developing countries, including Pakistan. Cases in the developed world are mostly migrants or travellers returning from third world countries. It is estimated 120 million infections and 700,000 deaths are caused annually by this disease worldwide[1]. The "first line drugs" used in the treatment of typhoid fever (ampicillin, chloramphenicol and cotrimoxazole) remained effective till the early 90s when plasmid mediated resistance emerged in Southeast Asia[2,3]. This lead to the use of fluoroquinolones, particularly ciprofloxacin, as standard treatment for typhoid, given their good safety profile and both extracellular and intracellular mechanism of action. However, from 2000 onwards, misuse, over the counter availability and selective pressure led to the emergence of *Salmonella* strains that were resistant to ciprofloxacin[4,5].

In 2004, the Clinical Laboratory Standards Institute (CLSI) validated the use of 30 µg Nalidixic acid disc as a screening method for determining resistance to fluoroquinolones in *Salmonella* (classical quinolone resistance) in laboratory antibiograms[6,7]. However it was seen that some strains were associated with slow or no response to fluoroquinolone treatment, suggesting that the Nalidixic acid disc did not detect all mechanisms of fluoroquinolone resistance (non-classical quinolone resistance). Consequently the CLSI revised its breakpoints in 2014, stating all strains sensitive to nalidixic acid (zone diameter ≥ 19mm) must have a ciprofloxacin zone diameter of ≥ 31mm using the 5 µg disc to be labeled as sensitive to the latter, while

all Nalidixic acid resistant strains should be reported as fluoroquinolone resistant as usual[8]. This study was done to find the frequency of fluoroquinolone resistant *Salmonella* in blood cultures using this new criterion published by the CLSI.

MATERIALS AND METHODS:

This study was conducted on all blood cultures received in the Microbiology laboratory, Sheikh Zayed Hospital, Lahore from 1st June 2014 to 30th May 2015. Blood cultures are routinely subcultured on enriched and differential media at this laboratory. *Salmonella* was identified by Gram staining of non-lactose fermenting colonies on differential media, oxidase negativity, biochemical testing including API 20E (Biomerieux, France) and slide agglutination with polyvalent and monovalent *Salmonella* antisera (Beckton Dickinson, USA). Susceptibility testing was done by the modified Kirby Bauer disc diffusion technique. Among other antimicrobials, 30 µg nalidixic acid disc (Oxoid, UK) and 5 µg Ciprofloxacin disc (Oxoid, UK) were tested. Using the CLSI 2014 breakpoints, strains that tested resistant to nalidixic acid were reported as resistant to ciprofloxacin. For nalidixic acid susceptible (zone diameter ≥ 19 mm) strains, the ciprofloxacin zone diameter was measured and ≥ 31 mm was reported as susceptible, 21-30 mm as intermediate and ≤ 20 mm as resistant.

RESULTS

A total of 41 *Salmonella* were isolated from 5273 blood cultures of which 35 were *Salmonella typhi* (85.3%) and 6 were *Salmonella paratyphi-A* (14.7%). Ciprofloxacin was resistant in 34/41 (82.9%) and susceptible in 7/41 (17.1%) strains. All strains were uniformly susceptible to cefotaxime, ceftriaxone, cefixime, meropenem/imipenem, and aztreonam. Ampicillin was resistant in 32/41 (78 %) strains. Chloramphenicol was intermittently available during this study period, but showed a 100 % susceptibility. Aminoglycosides were not tested as they may appear susceptible *in vitro* but are ineffective clinically in treating *Salmonella* infections and should not be reported as susceptible[8]. This is due to the fact that the organisms are located intracellularly and aminoglycosides have an extracellular mode of action[9].

Table 1: Antimicrobial susceptibility results of salmonella from blood cultures.

Antimicrobial		Susceptibility (%)	Resistance (%)
Ciprofloxacin	(05 µg)	17.1	82.9
Ampicillin	(10 µg)	22	78
Ceftriaxone	(10 µg)	100	-
Cefotaxime	(10µg)	100	-
Cefixime	(10 µg)	100	-
Imipenem	(10 µg)	100	-
Meropenem	(10 µg)	100	-
Aztreonam	(30 µg)	100	-

DISCUSSION

Currently more than 2500 serotypes of *Salmonella* have been identified including 1400 that are important in human disease causation[9]. The four serotypes that most commonly cause enteric fever are *Salmonella typhi, Salmonella paratyphi-A, B and C1*[9].

The introduction of chloramphenicol in 1948 greatly revolutionized the treatment of typhoid fever, however its common side effect of bone marrow aplasia and later development of plasmid mediated resistance necessitated the development of newer therapies such as amoxicillin and cotrimoxazole[10]. Since 1989, resistance to these agents in *Salmonella* strains also emerged in endemic areas[10,11]. Multidrug resistant strains (resistant to these "first line" antimicrobials) were increasingly reported from Pakistan, India, Middle East and Africa[11-14].

Fluoroquinolones, particularly ciprofloxacin, were introduced as treatment of typhoid fever in the early 90's. Due to their excellent safety profile and both intracellular and extracellular mode of action, widespread use soon became common. Reduced susceptibility to ciprofloxacin emerged due to indiscriminate use, over the counter availability and cheap formulations that delivered subclinical dosages. As far back as 1997, Vietnam reported quinolone resistance in *Salmonella typhi*[15,16]. The first case of fluoroquinolone treatment failure in

Pakistan was reported in 1993[17,18]. In 2004, CLSI recommended the use of the 30 µg nalidixic acid disc for reporting susceptibility or resistance to fluoroquinolones in laboratory antibiograms[6,7]. In 2011, Accou-Demartin et al reported *Salmonella* typhi strains with decreased susceptibility to ciprofloxacin that were susceptible to nalidixic acid (non-classical quinolone resistance)[2]. These strains were associated with fluoroquinolone treatment failure but could not be detected by the nalidixic acid screening test. Again, such strains were reported from the Indian Subcontinent and Southeast Asia[15,16,19].

Resistance to quinolones is caused by amino acid substitutions in the Quinolone Resistance Determining Region (QRDR) of DNA gyrase subunit *gyrA*, the target gene of quinolones. However, strains with non-classical quinolone resistance were seen to have mutations in *gyrB* subunit as well[2]. Other mechanisms may involve point mutations in *parC* and *parE* subunits encoding DNA topoisomerase IV[20].

In 2014, CLSI revised its ciprofloxacin breakpoints for reporting *Salmonella* susceptibility in clinical laboratories. Nalidixic acid resistant strains were to be reported as ciprofloxacin resistant as before, but nalidixic acid susceptible strains were to be evaluated separately for ciprofloxacin - a zone diameter of ≥ 31 mm was taken as susceptible, 21-30 mm as intermediate and ≤ 20 mm as resistant[8].

This study reported 82.9% of *Salmonella* strains resistant to ciprofloxacin following the new CLSI guidelines. A study in 2014 from Sindh reported 65.6% resistance using the CLSI 2010 guidelines[18]. A three year review by Aga Khan University, Karachi reported increase in fluoroquinolone resistance in *Salmonella* from 84.7 % to 91.7 % using CLSI 2009 guidelines ; 88.2% of *S. typhi* and 83.9 % *S. paratyphi-A* strains were resistant in that study[4]. In sharp contrast, a study at Children's Hospital, Lahore in 2012 reported 85.71 % susceptibility to ciprofloxacin[21]. Other studies in the past have also reported very low resistance rates[11,20]. The low rates could be attributed to the use of different testing mechanisms and/or to different interpretive criteria.

All strains in this study were susceptible to third generation cephalosporins, cefotaxime, ceftriaxone and cefixime. Other studies have also reported little or no resistance to these antimicrobials[1,4,11,18,20-22]. Ceftriaxone particularly has been used with success in the treatment of typhoid fever, even in ciprofloxacin resistant strains.

Ampicillin was resistant in 32/41 (78%) of the strains in this study. This frequency is close to a study from Children's Hospital, Lahore in 2012 which reported 76.2% resistance[21], and two others from Aga Khan Hospital, Karachi (66.1% resistance) [4] and Liaquat University, Jamshoro (82% resistance) [18].

The macrolide azithromycin in combination with ceftriaxone may prove to be effective in the treatment of *Salmonella* infection in areas where fluoroquinolone resistance is a problem and/or if the physician is reluctant to use quinolones such as in children.[23,24]

CONCLUSION

Fluoroquinolone resistance in *Salmonella* species is on the rise, and if combined with the problem of multi-drug resistance, will greatly limit treatment options for this organism. Misuse of antibiotics in our community is common, given over the counter availability, cheap formulations with the wrong dosage of active ingredient and oral formulations that are easy to self administer. This is a bigger problem than before and unless checked, may result in evolution of organisms resistant to currently available drugs. With the lethal Methicillin resistant *Staphylococcus aureus*, Vancomycin resistant Enterococci and Carbapenemase producing Enterobacteriaceae already established in healthcare settings and Multidrug resistant tuberculosis in the community, stricter antibiotic policies with routine surveillance must be introduced and implemented to ensure we remain adequately equipped in dealing with problematic infections.

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