Sjournals
Scientific Journals
Scientific Journal of Microbiology (2012) 1(4) 97-100

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

Antibiotic susceptibility profile for salmonella in the Buea health district

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ARTICLE INFO

Article history:
Received 17 September 2012
Accepted 25 September 2012
Available online 30 September 2012

Keywords:
Salmonella
Antibiotic
Sensitivity
Resistant
Culture
Agar

ABSTRACT

Resistance of *Salmonella* to antibiotics is a serious health problem in developing countries. The aim of this study was to determine the antibiotic susceptibility profile of Salmonella in Buea. Stool samples were collected from 100 patients presenting with symptoms of typhoid and cultured on different media. 21 samples were positive for Salmonella and antibiotic susceptibility testing was done on these samples by the disc diffusion method, using 9 different antibiotics. Out of the 21 samples, 18(85.7%) were sensitive to Ciprofloxacin while Nalidixic acid recorded a high rate of resistance with only 2(9.5%) being sensitive to the drug.

1. Introduction

Ampicillin and Co-trimoxazole, being cost effective and well tried primary drug of choice (Gautam et al., 2002), and Chloramphenicol were the first line drugs used as standard treatment for enteric fever until 1980 (Gopal et al., 2011). These first line drugs are abbreviated as ACC: Ampicillin, Co-trimoxazole and Chloramphenicol. Mirza et al. (1996) reported an emergence in resistance to chloramphenicol in *S typhi* in various parts of the world. The simultaneous resistance to three or more different groups of antimicrobials is defined as Multi Drug Resistance (MDR). Contributing factors to MDR in *S typhi* may be drug misuse, drug overuse and inappropriate prescribing practices by doctors among others [1]. MDR in *S typhi* has been reported in Egypt (El-Sherbins, 1996), Cebu (Virginia et al., 1997), Brazil (Fabiola et al., 2002) and India (Gautam et al., 2002; Gopal et al., 2011; Dhanashree, 2007). In

vitro experiments showed that fluoroquinolones had higher sensitivity and more effective clinical outcome against *Salmonella* than the ACC (Pegues et al., 2005). Since then, fluoroquinolones (Ciprofloxacin and Ofloxacin) are used as first line drugs (Gopal et al., 2011) instead of the ACC. It should be noted that high resistance of *Salmonella* to Nalidixic acid has been observed as: 92.5 % and 72.2 % respectively by Dhanashree, (2007) and Gopal et al. (2011). Gopal et al. (2011) also observed that *Salmonella* strains resistant to Nalidixic acid exhibit decreasing susceptibility to Ciprofloxacin, increasing the number of treatment failures (Dhanashree, 2007). The studies carried out by Dhanashree (2007) and Gopal et al. (2011) also emphasized the re-emergence of Chloramphenicol sensitive *Salmonella* strains and reducing susceptibility to Ciprofloxacin.

This study was therefore designed to determine the antibiotic susceptibility pattern of Salmonella isolates from patients who present with typhoid fever in the Buea Health District. So as to enable health care workers to make informed decisions as per the treatment regimen.

2. Materials and methods

100 people presenting with fever for at least a week and symptoms for typhoid in the Buea Regional Hospital were included in the study. The study was approved by the Faculty of Health Sciences ethics Committee of the University of Buea, Cameroon. The period of stool sample collection ran from April to July 2011. Signed informed consent was duly obtained from all participants prior to sample collection. The participant's medical records were reviewed to exclude those who were on antibiotic treatment for the past four weeks.

The stool samples from participants were collected in clean plastic stool containers and taken within one hour to the laboratory for analysis (Cheesbrough, 2006): samples were inoculated on selenite F broth overnight at 37^0C (Liofilchem). The specimens were sub-cultured on Salmonella Shigella (SS) agar and Eosine Methylene Blue (EMB) agar. *E coli* produced blue-black colonies with metallic sheen on EMB. Blood samples of patients who were positive for both *Salmonella* and *E coli* were not considered in the determination of antibody baseline titres given that there is cross reactivity between *Salmonella* and *E coli*. Samples which were positive for SS agar were subjected to biochemical tests on Kliger Iron Agar: base on the results of the culture on KIA, the various Salmonella species were identified. Afterwards, the urease test was performed to confirm Salmonella species: it should be noted that all salmonellae are urease negative. The antibiotic sensitivity test was only run for samples that were SS positive, had passed the KIA test and were urease negative. Mueller-Hinton agar was soaked and sterilised for 15 minutes. The medium was cooled to 45^0C and poured into plates and when the agar solidified, the plates were dried by flaming. A loop-full of a pure colony of confluent growth was suspended in sterile saline and the turbidity of the suspension was matched with turbidity standards. The plates were inoculated by dipping a sterile swab in the inoculum and streaking it all over the surface of the medium three times, rotating the plate through an angle of 60^0C after each application. The swab was then passed round the edge of the agar surface and the inoculum was left to dry for a few minutes with the lid closed. Sterile forceps were then used to place antibiotic discs on the inoculum, pressing each disc gently to ensure even contact with the medium. The plates were then incubated at 37^0C overnight within 30 minutes of preparation. After the incubation, the diameter of each zone measured and recorded in mm, interpreting the results according to critical diameters by comparing with standard tables [10].

2.1. Data analysis

Collected information and data were registered into a log book, and then entered into a software package, MINITAB 15 for data analysis. Descriptive statistics such as percentage and frequency were used in the description of sample characteristics.

3. Results

A total of 100 individuals wilfully participated in the study. They all presented with symptoms of typhoid. 59 (59%) were females while 41 (41%) were males. The ages ranged between 2 and 74 years with a mean age of 31.69 years.

21 cases were positive for *Salmonella* species. Among the 21 pathogens that were isolated from culture and identified by biochemical tests, 12 (57.1%) were *S paratyphi* A; 8 (38.1%) were *S typhi* and 1 (4.8%) was *S paratyphi* C.

For the antibiotic susceptibility test, nine antibiotics were used; these were: Co-trimoxazole, Chloramphenicol, Ceftriaxone, Ofloxacin, Norfloxacin, Gentamicin, Nalidixic acid, Ciprofloxacin and Amoxicilin. The highest susceptibility rate was observed with Ciprofloxacin (85.71%) and resistance of 9.52%. Ciprofloxacin was followed by Ofloxacin, Chloramphenicol, Norfloxacin, Co-trimoxazole, Gentamicin, Ceftriaxone, Amoxicilin and Nalidixic acid – having a high resistance level of 71.43% (Table 1).

Table 1
Comparison of the Sensitivities of the various antibiotics.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxicillin	13 (61.90)	5 (23.81)	3 (14.29)
Ciprofloxacin	18 (85.71)	1 (4.76)	2 (9.52)
Nalidixic Acid	2 (9.52)	4 (19.05)	15 (71.43)
Gentamicin	13 (65.00)	5 (25.00)	2 (10.00)
Norfloxacin	14 (66.67)	7 (33.33)	0(0.00)
Ofloxacin	16 (76.19)	4 (19.05)	1 (4.76)
Ceftriaxone	13 (65.00)	3 (15.00)	4 (20.00)
Chloramphenicol	15 (71.43)	2 (9.52)	4 (19.05)
Co-trimoxazole	14 (66.67)	2 (9.52)	5 (23.81)

4. Discussion

The overall prevalence of typhoid in this study was 21% following confirmation by stool culture and biochemical tests. This prevalence is similar to that reported by Ammah et al. (1999), 22% in Buea but different from that reported by Nsutebu et al. (2003), 2.5% in Tiko, Douala and Yaounde. This difference could be due to the fact that for the Widal test, Ammah et al. (1999) considered an antibody titre of 1/160 as diagnostic for typhoid while Nsutebu et al. (2003) considered a titre of 1/200.

The fluoroquinolones are presently used as the first line drugs against typhoid fever. Three fluoroquinolones were used in this study: Ciprofloxacin, Norfloxacin and Ofloxacin. These three antibiotics respectively had sensitivity percentages of 87.71%, 66.67% and 76.19% and their average sensitivity is 76.86%. This agrees with findings from Gautam et al. (2002) who had 71.5% but is slightly lower than that obtained by Gopal et al. (2011), 93.25%. This value though being high is on a decrease, indicating that regular exposure of the Salmonella microbe to fluoroquinolones brings about mutations on the site of action of these antibiotics. It can therefore be logically understood that this sensitivity might keep on dropping year after year. This drop could also be due to non-compliance of patients to the doctors' prescriptions and the sale of non-conventional drugs in the streets and markets. Also, decreasing sensitivity observed with the fluoroquinolones in general and Ciprofloxacin in particular could be due to high resistance rate observed with Nalidixic acid (a quinolone). Dhanashree (2007) and Gopal et al. (2011) reported decreasing susceptibility to Ciprofloxacin among Nalidixic acid - resistant Salmonella strains. In this study, Chloramphenicol and Co-trimoxazole that are part of the ACC had a combined sensitivity of 69.05%. Chloramphenicol alone had a sensitivity rate of 71.43%. This value is midway between results obtained by Gautam et al. (2002) and Gopal et al. (2011) but clearly shows an increase in susceptibility of *Salmonella* to the ACC as reported by Dhanashree (2007) and Gopal et al. (2011). This increase can be explained by the fact that as the ACC are not regularly used, strains that were resistant to ACC are slowly losing their resistance factors and therefore become susceptible to these antibiotics. As opposed to Gopal et al. (2011) who had sensitivity greater than 95%, this study, being consistent with Gautam et al. (2002) instead revealed a sensitivity of 65% for Ceftriaxone. The possible reasons for this are the ones mentioned above: drug misuse and non-compliance to physician's prescription. These reasons also account for the sensitivity of Gentamicin (65%); which is very low compared to the value of 98.85% gotten by Gopal et al. (2011).

The fluoroquinolones were found to be the best drugs for the treatment of typhoid; but there is also a re-emergence of Chloramphenicol susceptible Salmonella strains.

Acknowledgement

This study was funded through the Department of Medical laboratory Sciences departmental research grant.

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