The Susceptibility Pattern of Salmonella Species to Commonly Used Antibiotics in the Bamenda District Health Area, Cameroon

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Abstract The susceptibility of Salmonella bacteria to commonly used antibiotics such as ampicillin, chloramphenicol and trimethoprim/sulphamethoxazole is threatened by the emergence of resistance strains of Salmonella. A cross-sectional study was carried out from September 2014 to November 2014 in the Regional Hospital Bamenda on individuals presenting with symptoms of salmonellosis. The Salmonellae were isolated from stool by culturing in Salmonella-Shigella Agar and Kliger Iron Agar, the later in which the isolates produced specific biochemical characteristics which were conclusive. They were assessed for antimicrobial susceptibility by the disc diffusion method using Mueller-Hinton Agar following both CLSI and EUCAST manual instructions. A total of 253 samples were collected and 22 cases were positive for Salmonella species with a prevalence of 8.70%. The susceptibility of the isolated Salmonellae to seven antibiotics was noted with ciprofloxacin having an overall sensitivity of 52.38%, ofloxacin, 47.62%, ceftriaxone, 47.62%, and gentamicin, 38.10%. Chloramphenicol had a low sensitivity percentage of 28.57%, while co-trimoxazole and amoxicillin had a high resistance level of 100.00% (0% sensitivity). The fluoroquinolones were found to be the best drugs for the treatment of typhoid; but there was also a noticeable re-emergence of chloramphenicol susceptible Salmonella.

Keywords Salmonella; antibiotics; susceptibility; sensitivity; resistance; culture; agar

1. Introduction

Salmonella infection is a disease of humans and animals caused by organisms of the two species of Salmonella (Salmonella enterica and Salmonella bongori) [1]. It is an endemic disease in the tropics and subtropics. The disease is systemic and is often contracted by ingestion of food or water that is contaminated with the pathogen usually from a fecal-oral source. The occurrence of salmonellosis could also be an indicator of poor personal and environmental hygiene [2]. The illness may be mild or severe but sometimes fatal.

Infection with Salmonella has continued to be a major health problem despite the use of antibiotics and the development of newer antibacterial drugs. The prevalence of salmonellosis in Cameroon from the research study of 1999 was 22% [3] while that of 2003 was 2.5% [4]. The causative organism has rapidly gained resistance to antibiotics like ampicillin, ceftriaxone, and cotrimoxazole, and also to previously efficacious drugs like ciprofloxacin [5]. Although fluoroquinolones are drugs of choice to treat invasive Salmonella infections, decreased susceptibility to ciprofloxacin (DCS) is quickly increasing worldwide [6].

These observations with variations in the sensitivity patterns reported for most Salmonella species stress on the significance of continuous monitoring of antibiotic sensitivity patterns to provide suitable guidelines for treatment. Determining the antibiotic sensitivity pattern of isolates is necessary since it will guide the physicians in making the right choice of drugs when treating patients; thus ensuring quick treatment of the infection without aggravating the illness and preventing antibiotic resistance.

The aim of this study was to investigate the antibiotic sensitivity pattern of Salmonella spp. isolated from patients in the Bamenda District Health Area.

2. Patients and methods

2.1. Ethics statement

Ethical approval to conduct this study was obtained from the institutional ethical committee for research on human health of the School of Health Science, Catholic University of Central Africa and authorization from the Northwest Regional Delegation of Public Health. Samples were collected with authorization from the Bamenda Regional Hospital, Cameroon. Since this study meant handling of personal information, we had to comply with a number of important principles as set out in the Data Protection Act 1998. Informed consent was obtained from all study participants before they were enrolled in the study. Each
patient was given a code number and no identifiers were available on the dataset or the questionnaires.

2.2. Context and type of study
It was a prospective, descriptive cross-sectional study carried out at the Bamenda Regional Hospital. The sampling technique used was a nonprobabilistic method of convenience. Participants for the study were recruited systematical as they came for consultation in the hospital and were sent to the laboratory to do a Widal serology.

2.3. Study population
All patients of all age groups who had been consulted and requested to do typhoid serological test were included in the study. Pregnant women and patients under any form of antibiotic therapy were excluded from the population of the study.

2.4. Process of data collection
Stool samples were used for the isolation of the *Salmonella* spp. Stool samples from participants were collected in clean plastic stool containers, clearly labeled with codes, and taken within one hour to the laboratory for analysis. Other demographic information was taken down on questionnaires which carried the patients’ codes. Data for analysis was obtained after cultural isolation and antibiotic susceptibility tests.

2.5. Sample size determination
The sample size was calculated using the standard formula for sample size calculation $N = \frac{z^2pq}{d^2}$ (Lorentz’s formula). Where $z = \frac{1}{1.96}$ (which corresponds to a 95% confidence interval), $p = \frac{1}{10} = 0.05$, $q = 1 - p; d = 0.05$. Based on these, our minimum sample size was 254.93 patients.

2.6. Limitations of the study
This study was limited by the fact that samples had to be collected by the patients themselves without supervision in respect for the patients’ privacy, and thus contaminants could therefore be introduced by the patient, which could consequently hinder the isolation of the *Salmonella* species.

Sampling was limited to the Bamenda Regional Hospital. Further studies are encouraged in more hospitals within the Northwest Region in order to broaden the scope of investigations.

2.7. Quality control
After the preparation of the culture media, one uninoculated plate from each batch was incubated at 37 °C to ensure the quality of the plates. Failure of the plates to conform to standards which is less than five colonial growths per plate led to the elimination of the whole batch of prepared plates. *Escherichia coli* ATCC 25922 was used as negative control while *S. enterica serovar* Typhi MTCC was used as positive control for the effectiveness of the antibiotic discs.

2.8. Minimization of bias/errors
Patients’ samples were labeled with codes as soon as they were handed over to the technologists. Each sample could only be unencoded by verification on the questionnaires. This was done to ensure that results remained unidentifiable. After culture, plates and slants were verified by two technologists and positive samples were subcultured on the same Salmonella-Shigella Agar (SSA) to validate their positivity.

2.9. Culture and identification
Stool samples were used for the isolation of the *Salmonella* species (14). The stool samples from participants were collected in clean plastic stool containers, clearly labeled with codes and taken within one hour to the laboratory for analysis (6). Immediately after collection, 5–10 mL of sterile water was added to the stool samples and mixed. This was done to ensure proper sampling and to reduce the number of colonial growth on the streaked surface of the SSA. Samples were inoculated on SSA overnight at 37 °C. The SSA was prepared according to manufacturer’s instructions (Oxoid, Hampshire, UK). Samples which were positive for *Salmonella* on the SSA (clear or colorless colonies with black centers) were subjected to biochemical tests on Kliger Iron Agar (KIA). Based on the results of the overnight culture on KIA, the various *Salmonella* species were identified. They were identified by the following specific characteristics.

2.10. Susceptibility testing
The antibiotic sensitivity test was only run for samples that were SS and KIA positive. The sensitivity tests were carried out following the Clinical Laboratory Standards Institute (CLSI) guidelines and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) manual instructions by disc diffusion. Mueller-Hinton Agar was soaked and sterilized for 15 min. The medium was cooled to 45 °C and poured into plates and when the Agar solidified, the plates were dried by flaming. A loopful of a pure colony of confluent growth was suspended in sterile saline and the turbidity of the suspension matched with turbidity standard (0.5 MacFarland standard which corresponds to $1.5 \times 10^8$ CFU/mL) (6). 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° after each application. The swab was then passed round the edge of the Agar surface and the inoculum was left to dry for about 5 min with the lid closed. Sterile forceps were then used to place antibiotic
The following seven discs were used in this study: amoxicillin (25 μg/disc), chloramphenicol (30 μg/disc), cotrimoxazole (50 μg/disc), ciprofloxacin (30 μg/disc), ceftriaxone (30 μg/disc), ofloxacin (5 μg/disc), and gentamycin (10 μg/disc) which are commercially available 6 mm discs (Himedia laboratories). The plates were then incubated at 37 °C overnight within 30 min of preparation. After the incubation, the diameter of each zone was measured and recorded in millimeters, interpreting the results according to critical diameters by comparing with standard tables (6).

2.11. Statistical analysis

We used the statistical package for social sciences (SPSS) version 14.0 and EXCEL 2007 software to conduct a quantitative analysis of the data collected. The variables were summarized and examined. Simple descriptive statistics such as means, medians, modes, standard deviations, standard errors and proportions were used to describe the data as appropriate. Differences in proportions were calculated using mean differences and the corresponding 95% confidence interval (CI). Variables were cross-tabulated and hypotheses tested by applying appropriate statistical tests. We tested for association in categorical variables using the chi-squared ($\chi^2$) test, reporting corresponding $P$-values.

3. Results

3.1. Population and sample distribution

A total of 253 individuals participated in the study; 155 (61.26%) females and 98 (38.74%) males (Figure 1). Their ages ranged between one year and 80 years; with a mean age of 19.49 years.

3.2. Frequency of isolation

The age group with the highest number of specimens was the age group of less than 10 years (110/253). The next group with a high number of study participants was the age group between 21 years and 30 years with 52/253 patients.

A total of 22 cases were positive for Salmonella species out of the 253 tested giving it a prevalence of 8.70% (Figure 2). Salmonellosis prevails among school-aged children at a positivity rate of 36.36%.

3.3. Antibiotic susceptibility profile

The highest susceptibility rate was observed with ciprofloxacin (52.38%), followed by ofloxacin (47.62%), ceftriaxone (47.62%), and gentamycin (38.10%). Chloramphenicol had a low susceptibility percentage of 28.57% (Table 1), while co-trimoxazole and amoxicillin had a zero susceptibility rate which implies resistance level of 100.00% (Figure 3).

3.4. Discussion

The overall prevalence of typhoid in this study was 8.70% following confirmation by stool culture and biochemical tests. This prevalence is different from that reported by Njunda et al. [7], where a prevalence of 21% was reported in Buea, but a little higher than that reported by Nsutebu et al. [8] with a prevalence of 2.5% in Tiko, Douala and Yaoundé. These results are also lower than that obtained in other developing countries like Nigeria, 75% [9], but similar
to that obtained in India, 7.6% [10]. Our data indicate that salmonellosis is highly clinically relevant for school-aged children and young adults. The bacteria were isolated from children under 10 years of age at a positivity rate of 36.36%. This is similar to what was reported in the Democratic Republic of Congo [11,12] and Nigeria [9]. These results are in line with the World Health Organization (WHO) 2011 report in which it is clearly stated that the vast majority of typhoid fever cases occur in Asia, Africa, and Latin America where water borne diseases are highly prevalent, because of inadequate supply of potable water to the public, with concomitant poor environmental and personal hygiene.

The fluoroquinolones are presently used as the first line drugs against typhoid fever [13]. Two of the fluoroquinolones were used in this study: ciprofloxacin and ofloxacin. These two antibiotics had sensitivity percentages of 52.38% and 47.62%, respectively; and their average sensitivity is 50%. This differs from the findings of Gautam et al. in 2002 who had 71.5% [9] but yet lower than that obtained by Muthu et al. (2011), with a sensitivity percentage of 93.25% [10]. From the last study carried out by Njunda et al. (2012), we noticed a significant decrease in the susceptibility values in this study, from an average of 76.86% to an average of 50%. This drop could also be due to noncompliance of patients to the doctors’ prescriptions and the sale of nonconventional 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). Muthu et al. (2011) and Dhanashree (2007) reported decreasing susceptibility to ciprofloxacin among nalidixic acid-resistant Salmonella strains [4,10].

The fluoroquinolones were found to be the best drugs for the treatment of typhoid and decreased ciprofloxacin susceptibility (DCS) may lead to treatment failure and eventually fluoroquinolone resistance.

5. Conclusion
Salmonellosis continues to be an important global cause of infectious intestinal disease and in developing countries maintains its dominant position as one of the top three commonest causes of bacterial gastroenteritis.

The results of this study has further accenteduated the growing concern about the presence and the spread of multidrug resistant salmonellosis; thereby underscoring the need for the rational application of antibiotics and other necessary interventions that will help to control the menace of antibiotic resistance.

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Conflict of interest The authors declare that they have no conflict of interest.

References