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Full Length Research Paper

A study of the antibiotic resistant pattern of *Salmonella typhi* from some clinical samples

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Due to reported cases of antimicrobial resistance by many pathogenic bacteria against many antibiotics worldwide, and the sparse nature of antimicrobial resistance data, a retrospective study was carried out on 744 isolates of *Salmonella typhi* obtained from 974 samples from four different hospitals in Yola, Adamawa State, Nigeria between 2001-2004 to determine the resistance pattern of *S. typhi* to the most commonly used antibiotics cotrimoxazole, ciprofloxacin, chloramphenicol and ampicillin. High rates of resistance was found in most of the isolates studied. Resistance rates were 92.3, 88.8, 79.6, 53.5 and 20% to amoxicillin, ampicillin, chloramphenicol, cotrimoxazole and ciprofloxacin, respectively. The high percentage resistance to the antibiotics studied could be attributed to their prevailing usage and abuse in the area under study. The implication of the high percentage resistance recorded for the antibiotics is that only ciprofloxacin will effectively treat *S. typhi* infections. These results call for nationwide surveillance programme to monitor microbial trends and antimicrobial resistance patterns in Nigeria.

Key words: Antibiotics, resistance, surveillance, Nigeria, *Salmonella typhi.*

INTRODUCTION

Salmonella typhi is a bacterium that causes typhoid fever (enteric fever) . Typhoid fever is a global infection with a fatality rate of 10%. The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa), especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Anita et al., 2002; Doughari, 2005). The World Health Organisation (WHO) estimated an annual infectious rate of 21.6 million and approximate death rate of 600 000 with the highest percentage in Africa and Asia. Resistance to a number of antibiotics among *S. typhi* has become a serious problem. Strains of *S. typhi* resistant to chloramphenicol and other recommended antibiotics have been identified in several parts of Latin America, Asia and Africa (Threlfall et al., 2001; Benoit et al., 2003). Development of multi-drug resistance by the bacterium has further complicated the problem. Antibiotic resistance is further accelerated due to irrational use of antibiotics and over-the-counter purchase attitude by the populace, which is a very common phenomenon in Africa. Adequate documentation of resistant profiles of these organisms is also lacking (Alawode, 2003). Infections caused by resistant *Salmonella* and indeed other pathogens result in significant morbidity and mortality, and contribute to escalating healthcare costs worldwide. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem in many pathogens throughout the world (Pfaller et al., 1997; Keith and John, 2005). For practising physicians, clinical microbiologists and public health officials, knowledge of local antimicrobial resistance patterns is essential to guide empirical and pathogen-specific therapy. This information is also critical for optimal decisions regarding hospital formulary and infection control policies, for the rational formulation of public healthcare policies, and national and international research agendas in this area. Unfortunately, data regarding endemic antimicrobial resistance are unavailable in many parts of the world, especially from areas where over-the-counter antibiotic use is common. This work was therefore set out to investigate retrospectively, the antimicrobial resistant profile of *S. typhi* from clinical isolates against the most commonly prescribed antibiotics for its treatment in some hospitals

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Table 1. Frequency of *S. typhi* isolates from the stool samples examined in all the medical institutions studied.

Year	Number of specimens examined	Number of isolates (%) Occurrence of isolates)
2001	308	273 (88.6)
2002	263	187 (71%)
2003	196	103 (53.6%)
2004	207	181 (87.4%)
TOTAL	974	744 (76.4%)

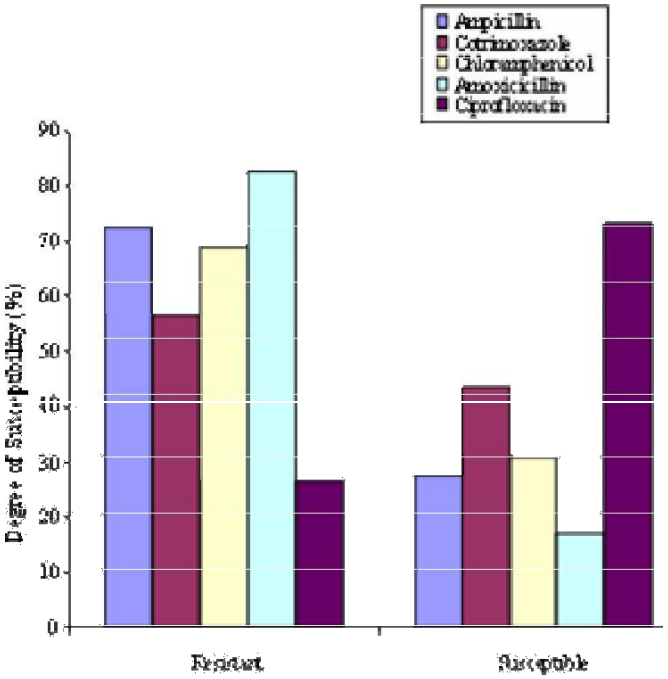

Figure 1. Antimicrobial resistance pattern of *S. typhi* against the most common antibiotics studied.

in this part of the world.

MATERIALS AND METHODS

Study design

The retrospective study was conducted utilizing microbiology laboratory records for four years (January 2001 to December 2004), from three hospitals - Peace Hospital, Freedom Polyclinic Hospitals, Specialist Hospital and one medical diagnostic laboratory–Biomedical Laboratory in Yola, Adamawa State, Nigeria. The hospitals and the medical diagnostic laboratory, collectively, attend to patients from all socioeconomic strata from Yola and the surrounding rural areas, although patient populations vary among individual institutions.

Microbiology Data

Microbiology records for the four years in these four institutions were reviewed for the purpose of this study. The isolates studied were confined to unrelated first isolates from different patients, and did not include multiple isolates from the same patient. All isolates were recovered from stool cultures. Specific antimicrobials tested varied from one institution to another. Information regarding the isolate, its source and antimicrobial susceptibility profile was collected and recorded. Data from different institutions were pooled.

Organism identification and susceptibility testing

All isolates were identified at the participating institution by standard laboratory methods (Pezzlo, 1992; Reisner et al., 1999). Each laboratory performed susceptibility testing according to their own standardized techniques based on current NCCLS guidelines (NCCLS, 1997). The Kirby-Bauer disc diffusion method using Nutrient agar plates, which is the predominant method employed, was used at all the hospitals and the medical laboratory studied.

The stool samples were generally streaked on deoxycholate citrate agar (DCA) and then subcultured on Salmonella-Shigella agar (SSA). The plates were all incubated at 37^{o}C for 24 h, after which the cultural and morphological characteristics of the isolates were studied. Identification of isolates was by standard microbiological methods, as described by Cheesbrough (1984). From the commercial antibiotic discs used for the susceptibility testing, results were pooled from those of ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg) and cotrimoxazole (10 µg), which are commonly used for the treatment of typhoid fever.

RESULTS AND DISCUSSION

Seven hundred and forty-four *S. typhi* isolates recovered from stool cultures of patients over the course of this 4-year retrospective study were analyzed. The frequency of isolation of *S. typhi* from the different specimens analyzed is given in Table 1. The result showed that of the entire 974 stool samples examined, 744 (76.4%) were positive for *S. typhi.* The rate of *S. typhi* occurrence ranged between 53.6% (in year 2003) to 273 (88.6%) in the year 2001. The result also revealed that the highest occurrence of *S. typhi* were in the years 2001 (88.6%) and 2004 (87.4%). The isolates were most resistant to amoxicillin (82.9%), followed by ampicillin (72.5%), chloramphenicol (69%) and cotrimoxazole (56.6%). Highest susceptibility of 73.4% (with lowest resistance of 26.6%) demonstrated against ciprofloxacin was recorded for all the isolates (Figure 1). A cursory look at the available records also showed multi-drug resistance among the isolates (result not shown). For instance in 2001, 62% of the isolates generally were resistant to both amoxycillin, ampicillin and chloramphenicol, while in 2002, 45% of the isolates were resistant to chloramphe- nicol and amoxycillin; and in 2003 57% of the isolates were resistant to amoxicillin.

The findings in this 4-year retrospective study at the three major hospitals and the Biomedical Laboratory in Yola, which revealed a high degree of antimicrobial resistance among the isolates studied, is very alarming. Out of

the total of 974 samples received in all the institutions, 744, representing 76.4%, yielded *S. typhi.* This confirms the clinical diagnosis, since all the samples were clinical isolates obtained from patients expressing symptoms associated with typhoid fever and were sent to the laboratory for investigation. This positive correlation of high infectious rate is, however, worrisome since it may indicate a high incidence rate in the area. The high infectious rate may not be unconnected with the poor state of hygiene in the State. Over-packed refuse dumps, as well as and blocked and eutroficating drainages with offensive emissions and human and animal excreta are common sites in major streets of the city. This picture is a common occurrence in the major cities of most developing countries, consequently food and drinking water sources and broken pipes are often easily contaminated by flies, cockroaches and rodents that scavenge for food in these contaminated environments, resulting in the high infection rate as observed from this study. The poor state of hygiene, low level of education and poverty affecting the greater part of the population, especially in the rural areas, are some of the contributing factors that make the inhabitants not to develop any interest in keeping a clean environment. Despite several efforts by the governments, such as declaration of specific days in a month for general township sanitation (in the major cities) and acquisition of typhoid vaccines, the aforementioned factors continue to be the major set-backs in the fight for eradication of typhoid fever in the developing countries, especially in Nigeria. In fact, so alarmed by the increased incidences of typhoid fever in the country, the Federal Government of Nigeria in 2003 acquired a large supply of anti-typhoid vaccines for vaccination of its citizenry (THE PUNCH, 2003).

The demonstration of a high rate of resistance by the isolates against amoxicillin, ampicillin, chloramphenicol and cotrimoxazole is a cause for concern. This is simply an indication that in the near future these antibiotics, which are the first line treatment for enteric fever, can no longer be used for the treatment of typhoid fever in this part of the world. Though no study on *S. typhi* was encountered during the cause of this research, other works on *S. typhi* also reported a high percentage of resistance against cloxacillin, ampicillin, erythromycin, penicillin, tetracycline, chloramphenicol, fluoroquinolones, macrolides and co-trimoxazole from other geographical areas (Archibald et al., 1997; Oyagade and Oguntoyinbo, 1997; Melo-Cristino, 1998; Mathai et al., 2001; Uba and Umar, 2002; Amani et al., 2003). Among staphylococci from 19 European hospitals, methicillin-resistance was found in 28 and 68% of *S. aureus* and coagulase-negative staphylococci, respectively (Schmitz et al., 1998).

The high rate of resistance observed from the results of this study is consistent with incidences of increased antibiotic resistance reported among Gram-negative bacilli such as *Klebsiella*, *Enterobacter*, *Citrobacter, Acinetobacter* and *Pseudomonas aeruginosa* in other parts of the world (Pfaller et al., 1998; Gales et al., 2001; Oplustil et al., 2001; Winokur et al., 2001). Because of high incidentces of antibiotic refractiveness by infectious bacteria, including *S. typhi,* many people, including even the urban dwellers, have turned to traditional herbs to seek for succor. The danger here is that most of these herbs are consumed without the knowledge of their toxicology; hence they may result in more serious complications. High susceptibility to ciprofloxacin is a welcome relief, since it is an indication of effectiveness of the antibiotic against the bacteria.

The variation in the sensitivity pattern and high resistant rate to these commonly used drugs could be attributed to the prevailing usage and abuse, and the common attitude of over-the- counter purchase of the drugs in the areas under study. This further suggests a relationship between antibiotic usage and the level of drug resistance encountered in this study.

The isolates represented both nosocomial- and community-acquired pathogens, since the patients from whom samples were collected in these four different health centres, come from different residential and working environments. Although data stratified by hospital are not presented in detail, resistance among Gram-positive cocci and Gram-negative bacilli was widespread between the participating hospitals. Epidemiological or clinical data were, however, not available to evaluate further the extent to which these resistance patterns reflect endemic antimic-robial resistance within the community versus nosocomial spread of resistant organisms within and between various hospitals. Nevertheless, these data highlight the fact that widespread antimicrobial resistance exists in the State (Adamawa, Nigeria). Resistance by microorganisms to antibiotics may be an indication of the presence of resis-tance factors such as R plasmids, and enzymes such as -lactamases, and of recent, extended - lactamase (ESBL) . The prevalence of ESBL enzymes has been incr-easing in many parts of the world. Infections caused by ESBL-producing isolates are difficult to treat, because they confer resistance to all currently available -lactam agents, except imipenem, and in some cases piperacillin-tazobactam (Jones et al., 1998; Winokur et al., 2001; Jones, 2001; Johnson et al., 2002). In addition, ESBL production is usually associated with resistance to other classes of antimicrobial agents, such as aminoglycosides and fluoroquinolones (Gales et al., 1997).

CONCLUSION

The data from this investigation suggests that antimicrobial resistance among *S. typhi* is common and significant in Yola, Nigeria. One of the explanations for these high resistance rates could be antibiotic usage in the respecttive institutions. Results of this study also have important implications for practising physicians with regard to empirical antibiotic selection. They also have important implications for authorities involved in hospital formulary deci-

sions, and in the development of policies regarding antibiotic utilization, infection control and public healthcare. The judicious use of antibiotics by health workers and efforts to control procurement and use of antibiotics officially in the locality will probably help to limit the increasing rates of drug resistance in pathogens. Our results call for further epidemiological studies to determine whether such isolates exist in the community and to determine whether extended spectrum ESBLs are highly endemic in the community and, on a larger scale, for the implementation of a regional and nationwide surveillance system to monitor antimicrobial resistance trends in Nigeria. The constant evaluation of the antibiotic-sensitivity pattern of pathogens for commonly used antimicrobial agents in a given locality should also be undertaken.

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