



Identification and Characterization of Antibiotic Resistant Bacteria Isolates from In-Patients at the Specialist Hospital, Sokoto, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JG, IGA and KJA gave the study concept and design and drafted the manuscript. Authors SBM, SBO and ND gave the study concept and design and performed data collection, analysis and interpretation. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was conducted to identify and characterize antibiotic resistant bacteria isolates from in-patients at Specialist Hospital, Sokoto, Nigeria.

Methods: A cross-sectional study was conducted among 245 patients with community-acquired infections, admitted into the various wards of the hospital over a period of 5 months. Blood, wound swab and urine samples were collected, transported and analyzed using standard procedures. Gram staining and biochemical characterization were conducted to identify bacteria isolates. Information on patients' bio-data and results of microbiological analysis were entered into a proforma, and analyzed using IBM SPSS version 17 statistical package.

Results: Forty nine (20.0%) of the 245 samples collected showed growth and were confirmed; the bacteria species isolated include *Staphylococcus aureus* (9.8%), *Escherichia coli* (6.5%), *Pseudomonas aeruginosa* (2.1%), and *Streptococcus pyogenes* (1.6%). There was no association ($p > 0.05$) between presence of positive samples and patients' age, sex or place of admission (ward). The overall sensitivity ranged from 69.4% for *Streptococcus pyogenes* to 79.2% for *Staphylococcus aureus*. Whereas *Streptococcus pyogenes* showed absolute sensitivity (100%) to Ampicillin and Tetracycline as compared to the other antibiotics used, it was completely resistant (0%) to gentamycin.

Conclusion: This study showed substantial occurrence of antibiotic resistant pathogens among patients with community-acquired infections in Specialist Hospital, Sokoto, Nigeria. Formulation and/or implementation of the necessary policy on safe antibiotic use; and organization of periodic educational enlightenment programmes for health workers and members of the public on prevention of antibiotic resistance through rational drug prescription and use are urgently needed.

Keywords: Identification; characterization; antibiotic resistant bacteria isolates; in-patients.

1. INTRODUCTION

The discovery of antibiotics used to treat human and animal diseases is one of the most significant public health accomplishments of the 20th century. However, other factors such as improvement in sanitation, nutrition and standard of living are as important as antibiotic therapy in the control and prevention of infectious diseases [1,2]. This success has also led to the development of bacterial resistance [3]. It was reported that microorganisms possess the ability to develop resistance to antibiotics soon after the introduction of antimicrobial therapy [4]. Recurring episodes of infection with multiple resistant organisms is one of such problems that have affected hospitals since the early 1950s when penicillin resistant *Staphylococci* initially emerged [4]. At the turn of this century, the World Health Organization estimated that infections accounted for 45% of deaths in Africa and cause 48% of premature deaths worldwide. It was also reported that bacteria cause a significant proportion of the infections in Africa [5]. Currently, there are new emerging antibiotic resistant bacteria trend, with about 70% resistant to at least one of the drugs most commonly used in treating infections [5]. This has resulted in not only increased morbidity and mortality but also in higher healthcare costs [6]. In Nigeria, indiscriminate use of drugs and self medication has resulted in the emergence of antibiotic resistant strains [7,8]. Reports from different parts of Nigeria have shown high prevalence of resistant strains of enteric organisms such as *E.coli* and *Shigella* species that were found to be resistant to antibiotics such as cotrimoxazole, tetracycline, chloramphenicol and ampicillin [9,10].

Information on the prevalent agents of bacterial infections and their resistance pattern would be invaluable in the choice of the most appropriate drugs in treating the prevalent infections; and also in the development of evidence based essential drug list. Little is known about the prevalent agents of bacterial infections and their resistance pattern in Sokoto, Nigeria, particularly among patients with community-acquired infections (CAIs). Community-acquired infections refer to infections contracted outside of a health care setting or infections present on admission [11]. This study was conducted to determine the antibiotic profile of specimens obtained from patients with CAIs admitted to the Specialist Hospital, Sokoto, Nigeria, with the broad objectives of establishing the resistant pattern of the organisms isolated from the specimens.

2. MATERIALS AND METHODS

2.1 Study Design, Sample Collection and Transportation

This cross-sectional descriptive study was carried out among patients with CAIs admitted into the various wards of the Specialist Hospital, Sokoto, Nigeria. The hospital is one of the two tertiary health institutions in Sokoto metropolis. It has a bed capacity of 570 and provides medical services to the residents in the metropolis, those referred from the other Local Government Areas in the state, as well as patients from the neighboring states (Kebbi and Zamfara) and country (Niger republic).

The sample size was estimated at 245 using the statistical formula for calculating sample size for cross-sectional descriptive studies [12], a 20%

prevalence of bacteria isolates from a previous study [13], and a precision level of 5%. The eligible study participants were selected by a 2-stage sampling technique. At the first stage 7 of the 15 wards in the hospital were selected by simple random sampling using the ballot option. At the second stage all the consecutively diagnosed patients with community acquired infections, that were admitted into the selected wards, and consented to participate were enrolled into the study by universal sampling over a period of 5 months until the required number of samples was obtained. Information on patients' bio-data and results of microbiological analysis were entered into a proforma. A total of 245 clinical samples were collected aseptically and transported as described by Elmer et al. [14], and Cheesbrough [15] to the Microbiology Laboratory of Usmanu Danfodio University Sokoto, Nigeria, for analysis.

2.2 Media Preparation and Isolation of Bacteria

All media used (Thioglycollate broth, McConkey agar, Blood agar, Chocolate agar, Mueller Hinton agar and Nutrient agar) were prepared according to manufacturer's specifications. Blood specimen collected from cases of bacteremia and septicemia were diluted with thioglycollate broth (1:10) and incubated at 37°C for 24 hours. Swab specimens from skin rashes and post-operative wounds were inoculated into the blood and chocolate agar; whereas, urine samples were centrifuged at 3000 rpm before inoculating the debris into the blood and chocolate agar media.

2.3 Biochemical Identification of Bacterial Isolates, Susceptibility Test and Determination of the Antibiotic Profile of Resistant Bacteria Isolated

Isolates were subjected to Gram staining and biochemical tests (catalase, coagulase, motility, oxidase, urease, methylred Voges Prouskauer indole, Bacitracin and sugar utilization) as described by Cowan and Steel [16], Cheesbrough [15], and Oyeleke and Manga [17]. The bacteria isolates were tested for antibiotic susceptibility by the method described by Elmer et al. [14]. An overnight broth culture of the test organism was diluted (1:100) to obtain approximately 1.5×10^9 /ml, by comparing its turbidity to McFarland turbidity standard scale 5.

Commercially prepared antibiotic discs: Ampiclox (10 mcg), Chloramphenicol (25 mcg),

Streptomycin (10 mcg) and Ciprofloxacin (30 mcg) (Biotec-UK) were used. The Kirby-Bauer disk diffusion method was adopted in determining the antibiotic profile of the isolated resistant bacteria [14].

2.4 Data Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 17 statistical computer software package. Frequency distribution tables were constructed; and cross tabulations were done to examine relationship between categorical variables. Chi-square test of independent association was used to test for relationship between categorical variables. All levels of significance were set at $p < 0.05$.

2.5 Ethical Consideration

Ethical clearance was obtained from the Ethical Committee of Sokoto State Ministry of Health, Sokoto, Nigeria. Permission to conduct the study was obtained from the Management of the Specialist Hospital, Sokoto, Nigeria. Informed consent was also obtained from the patients before collection of samples.

3. RESULTS AND DISCUSSION

3.1 Distribution of Patients by Age and Sex

A larger proportion 60 (24.5%) of the 245 patients from which the samples were collected were in the 31 – 40 years age group, followed by those in the 21 – 30 years age group (20.4%); and majority of the patients were females (55.1%) as shown in Table 1.

Table 1. Distribution of samples by age and sex of patients

Variables	Frequency (%) n = 245
Age group (in years)	
≤10	30 (12.2)
11-20	45 (18.4)
21-30	50 (20.4)
31-40	60 (24.5)
41-50	35 (14.3)
≥ 51	25 (10.2)
Sex	
Male	110 (44.9)
Female	135 (55.1)

3.2 Prevalence and Pattern of Bacteria Isolates

Forty nine (20.0%) of the 245 samples collected showed growth and were confirmed; the most common bacteria species isolated were *Staphylococcus aureus* 24 (9.8%), and *Escherichia coli* 16 (6.5%). Other bacteria species isolated include *Pseudomonas aeruginosa* 5 (2.1%) and *Streptococcus pyogenes* 4 (1.6%) as shown in Table 2. The pattern of bacterial isolates in this study is similar to the finding in studies conducted among patients with community acquired-infections in other places including Kano, Nigeria [18], Jimma, Ethiopia [19], Bahia, Brazil [20] and Tehran, Iran [21], with *Staphylococcus aureus* and *Escherichia coli* being among the most common pathogens isolated in these studies.

Larger proportions of positive samples were obtained among participants in the 31 -40 years (4.9%) and 41 – 50 years (4.08%) age groups,

but there was no significant difference in the distribution of bacteria isolates by patients' age ($\chi^2 = 0.1133$, $p > 0.05$) as shown in Table 3.

Table 2. Prevalence and distribution of bacteria isolates among patients

Bacterial specie	Frequency (%) n = 245
No bacteria isolated	196 (80.0)
<i>Staphylococcus aureus</i>	24 (9.8)
<i>Escherichia coli</i>	16 (6.5)
<i>Pseudomonas aeruginosa</i>	5 (2.1)
<i>Streptococcus pyogenes</i>	4 (1.6)

A slightly higher proportion of positive samples were obtained among males (10.2%) as compared to females (9.8%), but there was no significant difference in the distribution of bacteria isolates by patients' sex ($\chi^2 = 0.6034$, $p > 0.05$). *Staphylococcus aureus* had the highest occurrence of 4.5% and 5.3% in both male and female patients (Fig. 1).

Table 3. Distribution of the bacteria isolates by patients' age

Age group (in years)	Number of samples (%)	Bacteria isolated (%)
≤ 10	30 (12.2)	3 (1.2)
11-20	45 (18.4)	7 (2.9)
21-30	50 (20.4)	9 (3.7)
31-40	60 (24.5)	12 (4.9)
41-50	35 (14.3)	10 (4.1)
≥ 51	25 (10.8)	8 (3.2)
Total	245 (100)	49 (20.0)

$$\chi^2 = 0.1133, p > 0.05$$

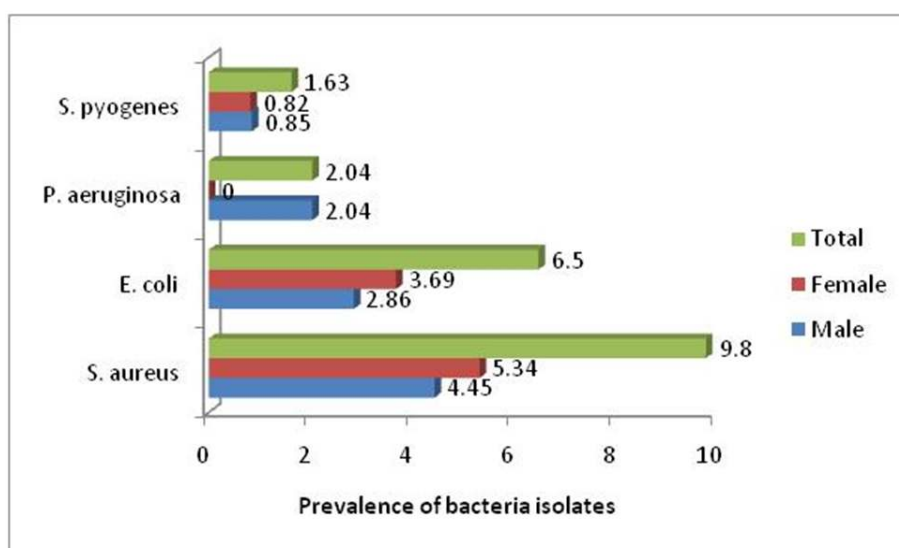


Fig. 1. Distribution of bacteria isolates by patients' sex

Also, larger proportions of positive samples were obtained among patients admitted at Female Medical Ward (4.9%) and Accident and Emergency Unit of the hospital (3.7%), but there was no significant difference in the occurrence of bacteria isolate by patients' place of admission ($\chi^2 = 0.257$, $p > 0.05$) as shown in Table 4.

The high prevalence of bacteria isolates (20.0%) among the patients in this study could be due to the fact that 30% of healthy individuals harbor these bacteria in their moist epithelium [22]. This may probably account for the occasional involvement of the bacteria in the cause of bacteremia or/and presence in the blood of patients especially the immune-compromised individuals [22]. The result of this study also agrees with the findings of Sean and Pitman [23], in which *Staphylococcus aureus* and *Streptococcus Pyogenes* were the most common bacteria isolated from clinical samples collected from sick patients in a hospital studied; and if this trend is left unchecked, it could result to serious public health implications. The cost of healthcare for patients with resistant infections is known to be higher than care for patients with non-resistant infections due to longer duration of illness, additional tests and use of more expensive drugs. In addition, it is evident that without effective antibiotics, the success of major surgery and cancer chemotherapy would be compromised [24].

Among the bacteria isolates tested for susceptibility profile against Ampicillin, Cotrimoxazole, Gentamycin and Tetracycline, the overall sensitivity ranged from 64.9% to 79.2%. Only *Streptococcus pyogenes* showed absolute (100%) sensitivity to Ampicillin and Tetracycline (Table 5) when compared to the other agents used, but it was completely resistant (0%) to Gentamycin. This finding is in agreement with the report of Shankar et al. [25], in which a similar observation was made. Studies conducted among patients with community-acquired infections in other places also reported high rates of antimicrobial resistance to the antimicrobial agents commonly used as empiric therapy for the prevalent CAIs in the respective study areas [18-21]. Whereas the absolute (100%) sensitivity of *Streptococcus pyogenes* to Ampicillin and Tetracycline is re-assuring, the zero sensitivity to Gentamycin observed is threatening as it points to development of resistance to the commonly used antimicrobial agents in the study area; and the dire consequences in terms of poor response to medication, extended hospital stay and high cost of treatment. These findings suggest the urgent need for Government to urgently formulate and/or implement the necessary policy on safe antibiotic use; in addition to organization of periodic educational enlightenment programmes for health workers and members of the public on prevention of antibiotic resistance through rational drug prescription and use.

Table 4. Occurrence of bacteria isolates by patients' place of admission

Ward	Number of samples (%)	Bacteria isolated (%)
Female Medical	37 (15.1)	12 (4.9)
Male Medical	35 (14.3)	7 (2.9)
Accident and emergency	44 (18.0)	9 (3.7)
Pediatric	38 (15.5)	5 (2.0)
Post-natal	29 (11.8)	4 (1.6)
Female surgical	30 (12.2)	7 (2.9)
Male surgical	32 (13.1)	5 (2.0)
Total	245 (100)	49 (20.0)

$$\chi^2 = 0.257, p > 0.05$$

Table 5. Sensitivity pattern of bacteria isolated from patients

Bacteria isolates	Antibiotics sensitivity pattern (%)				
	AP No (%)	COT No (%)	GEN No (%)	TET No (%)	ORS Percent
<i>S. aureus</i> (n = 24)	20 (83.3)	15 (62.5)	23 (95.8)	18 (75.0)	79.20
<i>E. coli</i> (n = 16)	11 (68.8)	8 (50.0)	14 (87.5)	12 (75.0)	70.30
<i>P. aeruginosa</i> (n = 11)	9 (81.8)	8 (72.7)	10 (90.9)	7 (63.6)	77.30
<i>S. pyogenes</i> (n = 9)	9 (100.0)	7 (77.8)	0 (0)	9 (100)	69.40

Key: AP = Ampicillin, COT = Cotrimoxazole, GEN = Gentamycin, TET = Tetracycline;
ORS = Overall sensitivity

4. CONCLUSION

This study showed substantial occurrence of antibiotic resistant pathogens among patients with community acquired infections in Specialist Hospital, Sokoto, Nigeria. Formulation and/or implementation of the necessary policy on safe antibiotic use; and organization of periodic educational enlightenment programmes for health workers and members of the public on prevention of antibiotic resistance through rational drug prescription and use are urgently needed.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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