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Research Article

Antibiotics Susceptibility Profile of Gram-Positive Bacteria from Primary Health Centers in Jega, Kebbi State

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Abstract

Nosocomial or healthcare-associated infection (HCAI) is an infection acquired during receiving health care that was not present during admission. The research aimed to determine the antibiotic susceptibility pattern of gram-positive bacteria isolated from Primary Health Centers in Jega Town. A total of fifty (50) swab samples were collected from 10 different health centers and analyzed using the streak plate technique. Pure bacterial isolates were maintained and characterized using biochemical tests; their percentage of occurrence show; *Staphylococcus aureus* 18 (43.9%), *Enterococcus feacalis* 8 (19.5%), *Streptococcus spp* 8 (19.5%), *Bacillus cereus* 4 (9.8%), and *Staphylococcus epidermidis* 3 (7.3%). McFarland standard solution was prepared and used to control inoculants, after which the antibiotic susceptibility pattern of the isolates was determined using the disc diffusion method. *Staphylococcus epidermidis* was resistant to Gentamycin, and other isolates were multi-drug resistant. In light of this research, there is a need for thorough disinfection and conscientious contact control procedures to minimize the spread of these pathogens in health centers where interaction between patients, HCWs, and caregivers is widespread and frequent.

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INTRODUCTION

Nosocomial or healthcare-associated infections (HCAI) appear in a patient under medical care in the hospital or other healthcare facility which was absent at the time of admission[1]. These infections can occur during healthcare delivery for other diseases and even after the discharge of the patients. Additionally, they comprise occupational infections among the medical staff. Invasive devices such as catheters and ventilators employed in modern health care are associated with these infections[2]. With much medical equipment in hospitals coming in direct contact with healthcare workers, patients, technicians, cleaners, and sometimes caregivers, it is essential to pay close attention to their capacity to harbor potentially harmful pathogens[3].

Nosocomial infections affect many patients globally, leading to increased mortality and financial impact on healthcare systems[4]. While the actual global burden of healthcare-associated infection (HAI) remains unknown due to the lack of reliable data and surveillance systems. The endemic burden of HAI appears to be higher in developing countries[5]. A pooled analysis of data from developing countries showed an HAI prevalence of 15.5%, most of which occur as ventilator-associated pneumonia (VAP) and neonatal infections in intensive care settings[6].

According to the Extended Prevalence of Infection in Intensive Care (EPIC II) study, the proportion of infected Patients within the ICU is often as high as 51%[7]. Out of every hundred hospitalized patients, 7% in developed and 10% in developing

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countries can acquire one of the healthcare-associated infections[1]. Healthcare-associated infection (HAI) complications are broad and depend on the type of infection, the severity of illness, and implicated pathogen. The most prevalent nosocomial infections were pneumonia, urinary tract infection, bloodstream infection (BSI), and meningitis/ventriculitis[8]. Significant independent associations with pneumonia included older age, poor Hunt and Hess grade, intubation/mechanical ventilation, and loss of consciousness *at ictus*[9].

Identifying common fomites and associated pathogens in any hospital setting is essential because the most critical factor in preventing disease is simply identifying what has been transferring the disease in the first place[10]. Fomites are, therefore, an opportunity to interrupt the spread of infection by recognizing them, avoiding them, disinfecting them, or cleansing the hands after touching them; the spread of many infections can be halted[11]. Ikeh and Isamade[12], in their study of bacterial flora of fomites in a Nigerian multi-disciplinary intensive care unit, reported that the majority of the isolates were Gram-positives organisms (52.2%: 12/23) as compared to the Gram-negative (47.8%: 11/23).

Staphylococci were isolated from all the fomites; *Staphylococcus epidermidis* (8.7%: 2/23) from the IVF stand, stethoscope, and *Staphylococcus aureus* (21.7%: 5/23) from the other fomites. Isolation of more Gram-positive organisms is consistent with previous reports[13,14] and agrees with Inweregbu *et al*.[15] and Ikeh and Isamade[12] that Gram-positive bacteria have overtaken the Gram-negative as the predominant bacteria isolated from fomites. Gram-positive organisms have earlier been noted by Gopinathan *et al*.[16] to be causing more severe infections than ever in surgical patients, who are increasingly aged, ill, and debilitated.

The present study necessitates investigating the constant drug resistance in the area, as the number of prevalent cases is frequently referred to the general hospital. It is also discovered that people are contracted some diseases that are not transported from their homes but within the hospital settings, where proper environmental sanitation is not practiced. This study was therefore conducted to isolate Gram-positive bacteria (more prominent for nosocomial infections) from Primary Health Centers in Jega and to determine their antibiotic susceptibility pattern to the commonly prescribed antibiotics in primary health centers.

MATERIALS AND METHODS

Research area/Sites

The research area was Jega Local Government Area (**Figure 1**), located at the latitude 12.3667°N and longitude 4.6333°E, with a total area of 891 km^2 and a population of 193,352 as of the 2006 Nigeria census estimate. The area has one General Hospital and 11 Primary Health Cares (PHC) that work daily to safeguard the health status of the inhabitants, predominantly Gimbanawa, with minority groups of Kambari, Zamfarawa, and Zabarmawa. The samples were collected at PHC Birnin Yari, PHC Tudun Wada, PHC GRA, PHC Town Council, PHC Jandutsi, PHC Basaura, PHC Bumbegu, PHC Gindi, PHC Nassarawa, and PHC Kimba.

Sample collection

A total of 50 samples from fomites were collected from ten distinct Primary Health Centers (five samples each); this was aseptically performed using sterile swab sticks as described in the report of Olise and Simon-Oke[17].

Isolation of bacterial isolates

The swab samples were cultured on Mannitol Salt Agar medium using the streak plate technique to select only Gram-positive bacteria. The incubations were demonstrated at 37°C for 18-24 hours; isolates were sub-cultured and further maintained on Nutrient Agar slants[18].

Biochemical identification of bacterial isolates

Citrate utilization, Indole, Methyl red, Voges–Proskauer, Triple sugar ion, Catalase, and Coagulase tests were employed to determine the biochemical reaction of the isolates[19].

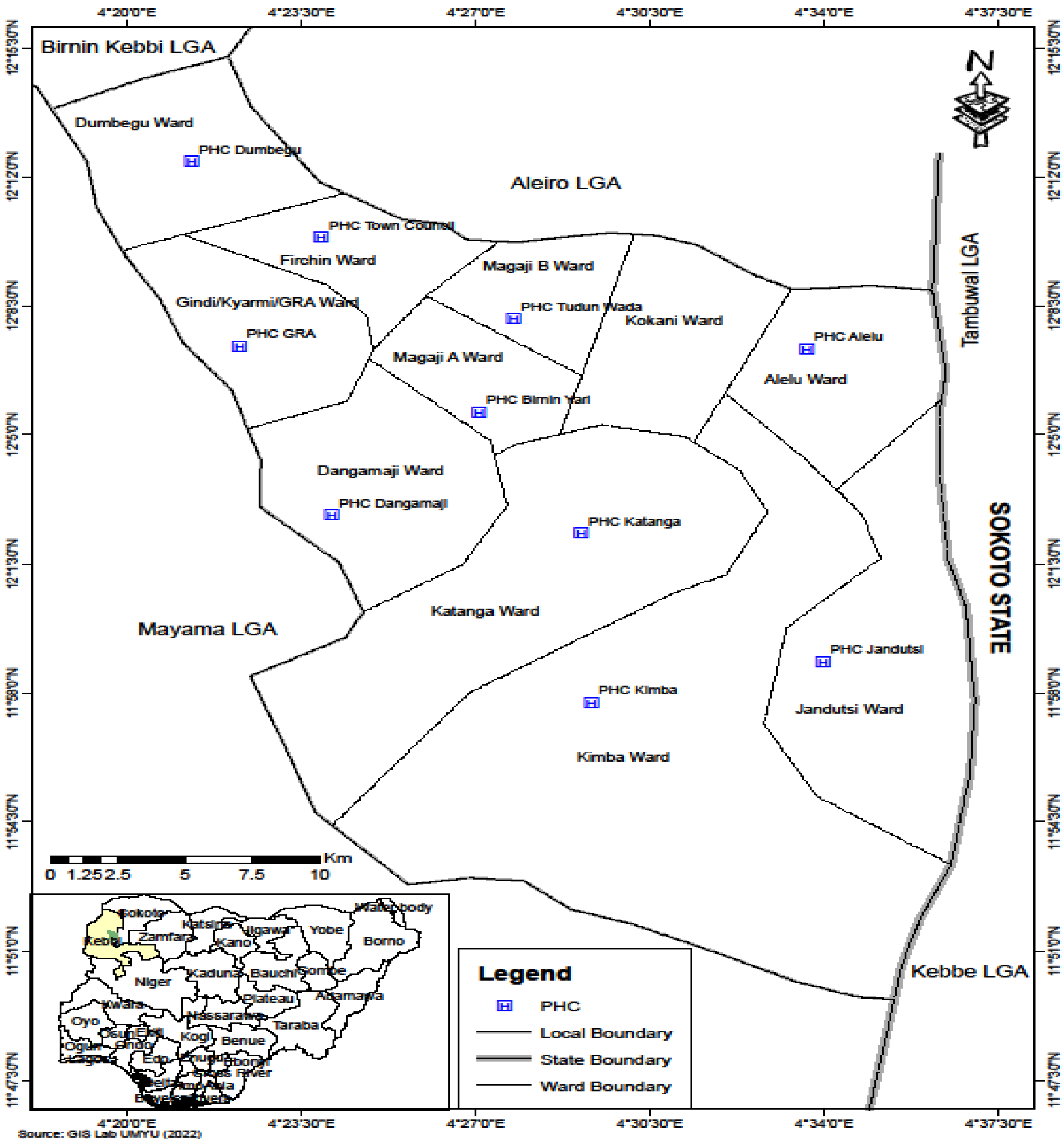

Figure 1. Map of the research area indicating different sampling sites.

Antibiotic susceptibility testing

The antibiotics susceptibility profile of the isolates was determined using the disc diffusion method, the antibiotic discs used were those of Maxi disc; Septrin (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), ciprofloxacin (30 µg), amoxicillin (30 µg), augmentin (10 µg), gentamycin (30 µg), pefloxacin (30 µg), Tarivid (10 µg), streptomycin (30 µg). The McFarland standard was prepared by adding 1 mL of sulphuric acid into 99 mL of water and 0.5 g of dehydrated barium chloride into 50 mL of distilled water, each in a separate conical flask mixed. About 0.6 mL of barium chloride solution and mixed. The small volume of the solution was transferred into the Bijou bottle and was used to control inoculants. Aliquots of 100 µL from each suspension were spread-plated on Mueller-Hinton Agar plates and incubated at 37°C for 6 hours. Antibiotic discs were applied to the plates using sterile forceps, and the plates were incubated at 37°C for 24 hours. The zone of inhibitions of the plates was measured and classified as resistant (R), intermediate (I), and or sensitive (S) to a particular antibiotic using standard reference values according to the Clinical Laboratory Standards Institute (CLSI)[20].

Data analysis

All the experiments were performed in triplicates, and the data obtained were analyzed as mean plus standard deviation. The statistical analysis was performed using the analysis of variance (ANOVA), and the least significant difference between means and standard deviation was expressed using SPSS version 20.0.

RESULTS AND DISCUSSION

Isolation and identification of bacteria

The biochemical identification of the isolates and their occurrence (**Figure 2**) revealed five bacterial genera; *S. aureus* 18 (43.9%), *Enterococcus feacalis* 8 (19.5%), *Streptococcus sp* 8(19.5%), *Bacillus cereus* 4 (9.8%) and *S. epidermidis* 3 (7.3%). The occurrence of the bacteria could probably be because they are members of the body flora of both asymptomatic carriers and sick persons. These organisms can be spread by the hand, expelled from the respiratory tract, or transmitted by animate or inanimate objects[21]. Their primary source(s) of colonization on the fomites might likely be nasal carriage by hospital personnel[22], facilitated by hand-to-mouth or hand-to-nose contact while using these fomites, and poor hand-washing habits[23]. This finding is consistent with the reports of Neely and Maley[13] as well as Chikere *et al.*[24] and agrees with Inweregbu *et al.*[15] that Gram-positive bacteria have overtaken the Gram-negative as the predominant bacteria isolated from hospital fomites. Gram-positive organisms have earlier been noted to be causing more severe infections than ever in surgical patients, who are increasingly aged, ill, and debilitated[25].

The highest occurrence of *S. aureus* from almost all the samples shows its ubiquity in nature and that they can be sources of infection in patients, as previously noted by previous research[12,15]. Although the strains of the isolated *S. aureus* were not determined in this study, methicillin-resistant *Staphylococcus aureus* (MRSA) strains are transmissible from many fomites to the skin. For example, earlier studies showed that one in three stethoscopes tested to harbor *S. aureus* and that 15% of all stethoscopes tested were contaminated with MRSA[26]. *Staphylococcus epidermidis* was isolated with the lowest frequency in this study. Though these strains are known to be non-pathogenic to the body, when they harbor antimicrobial resistance genes, they constitute a severe health hazard. This *S. epidermidis* has been isolated from keyboards on multiple user computers[27], and increased virulence of this organism resulting from the acquisition of methicillin-resistance has been recognized[28].

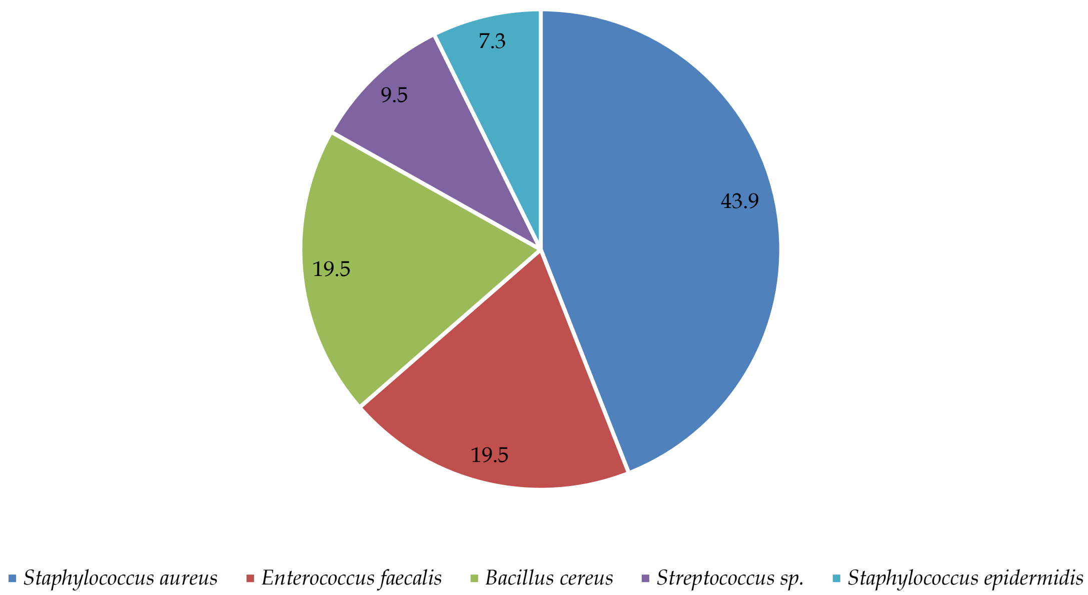

Figure 2. Percentage occurrence of the identified bacteria.

Antibiotic susceptibility profile of the bacterial isolates

The diameter of the zone of inhibition of the antibiotics (**Table I**) revealed that all the bacterial isolates were susceptible to streptomycin (S) and ciprofloxacin (CPX) at ≥25.0±0.03 mm, with the intermediate pattern on sparfloxacin (SP), amoxicillin (AM) and Tarivid (OFD) at ≤ 24.0±0.0 mm, while all the bacterial isolates exhibit resistance to augmentin (AU) and gentamicin (CN) at ≤ 16.0±0.05 mm, with amoxicillin (AM) showing resistance to *S. aureus*, *E. feacalis*, and *Strep. sp*. at ≤ 16.0±0.05mm. The susceptibility of bacterial isolates to antibiotics (**Figure 3**) indicates the ineffectiveness of the AU, CN, and AM in treating HAIs that might result from infection with these pathogens.

This implies that these fomites might act as vehicles for transferring these pathogens. Many studies have shown uniforms to be potential reservoirs for hospital organisms, potentially reinfecting the hands of HCWs, thereby causing resistance patterns to antibiotics[23]. Treakle *et al*.[29] showed a large proportion of HCWs' white coats to be contaminated with *S. aureus*, including MRSA and postulated that white coats might be an essential vector for patient-to-patient transmission of *S. aureus*. Potential pathogens such as *S. aureus*, *Acinetobacter* spp., and enterococci have been recently isolated from hands that were used to touch uniforms[23].

The resistance of the isolates is in line with the findings by Sani *et al*.[30] and Ogunshe *et al*.[31], who reported gram-positive isolates to demonstrate a low-to-moderate sensitivity to gentamicin (20.0–57.1%), streptomycin (21.7–57.1%), and ciprofloxacin (20.0–42.9%); a moderate sensitivity to ceftriaxone (57.1–60.9%); and high sensitivity to imipenem (85.7–87.0%) except for *S. epidermidis* which showed a low sensitivity (20.0%) to imipenem. The Gram-positive cocci were least resistant to imipenem and ceftriaxone, with the isolates from acute wounds demonstrating slightly higher resistance.

Chikere *et al*.[24] reported a similar weakness and activity of some antibiotics against bacteria from clinical specimens; as more bacteria become resistant to antibiotics, the ability to control the spread of these bacteria with antibiotic treatments decreases. These findings also align with the investigation of Lee *et al*.[32], who found a strong correlation between oxacillin resistance and co-resistance to non–β-lactam antimicrobials such as gentamicin, erythromycin, and ciprofloxacin ($p > .01$). Such high rates of co-resistance suggest the presence of multidrug-resistant MRSA strains. The strong resistance of the augmentin (AU), gentamicin (CN), and amoxicillin (AM) obtained agreed with the findings of Sani *et al*.[30] and Ogunshe *et al*.[31]. in their studies on antibiotic susceptibility of wound swab isolates in a tertiary hospital in southwest Nigeria and microbiological evaluation of antibiotic resistance in bacterial flora from skin wounds respectively.

Table I. Mean ± standard deviation of the zone of inhibition (mm) of the antibiotics to the bacterial isolates

Antibiotic	Potency (µg)	*S. aureus*	*E. feacalis*	*B. cereus*	*Strep. sp*	*S. epidermidis*
SXT	30	26.0^{a}±0.0	21.0^{b}±0.3	24.0^{ab}±0.0	30.0^{a}±0.3	28.0^{a}±0.3
CH	30	30.0^{a}±0.3	28.0^{a}±0.4	22.0^{b}±0.02	28.0^{a}±0.0	30.0^{a}±0.3
SP	10	25.0^{a}±0.0	28.0^{a}±0.4	25.0^{a}±0.03	26.0^{a}±0.1	25.0^{a}±0.2
CPX	30	30.0^{a}±0.4	30.0^{a}±0.3	30.0^{a}±0.3	30.0^{a}±0.3	31.0^{a}±0.3
AM	30	14.0^{a}±0.0	2.0^{b}±0.0	20.0^{c}±0.0	16.0^{ac}±0.05	25.0^{d}±0.1
AU	10	5.0^{a}±0.0	00.0^{b}±0.0	00.0^{b}±0.0	5.0^{a}±0.0	00.0^{b}±0.0
CN	30	11.0^{a}±0.0	8.0^{ab}±0.01	5.0^{b}±0.0	00.0^{c}±0.0	6.0^{b}±0.0
PEF	30	31.0^{a}±0.2	30.0^{a}±0.3	28.0^{ab}±0.1	26.0^{b}±0.1	25.0^{b}±0.1
OFD	10	21.0^{a}±0.5	26.0^{b}±0.1	25.0^{b}±0.2	21.0^{a}±0.3	22.0^{a}±0.02
S	30	32.0^{a}±0.6	30.0^{a}±0.0	30.0^{a}±0.3	32.0^{a}±0.03	30.0^{a}±0.0

Mean values with different alphabet appearing on the same row are significantly different otherwise they are the same

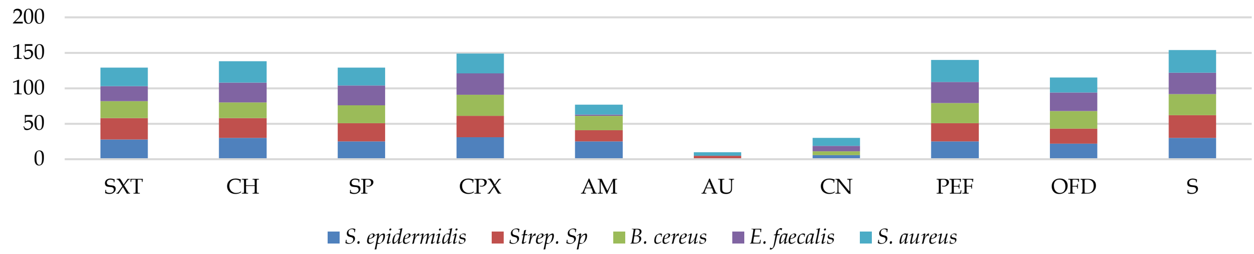

Figure 3. Antibiotic susceptibility pattern of the bacterial isolates. SXT= Septin, CH= chloramphenicol, SP= sparfloxacin, CPX= ciprofloxacin, AM= amoxicillin, AU= augmentin, CN= gentamycin, PEF= pefloxacin, OFD= Tarivid, S= streptomycin.

CONCLUSION

Based on the study findings, it is concluded that the hospital equipment (Benches, beds, stethoscopes, door handles, tables, cupboards, and chairs) harbored infectious pathogens with the potential of causing hospital-acquired infections (HAIs), which may eventually lead to diseases like urinary tract infections (UTIs), gastroenteritis, meningitis, pneumonia, septicemia, endocarditis and or wound infections. In light of this, there is a need for thorough disinfection and conscientious contact control procedures to minimize the spread of these pathogens in health centers where interaction between patients, HCWs, and caregivers is ubiquitous and frequent.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

No conflict of interest among the study participants exist.

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