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Antibiotic Resistance in Nigerian Hospitals: Phenotypic Evidence, Genetic Drivers, and Control Implications in Antimicrobial Stewardship

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ABSTRACT

Antibiotic resistance of bacteria agents *(Staphylococcis aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pyogenes*) of nosocomial wound infections, were assessed by in vitro susceptibility testing, evaluating the possible drug interactions among several antibiotics and the plasmid profile of DNA of isolate. Ninety-six clinical isolates of (*S.aureus, P aeruginosa, E coll and S pyogenes)* obtained from two different Hospitals, were collected. Suceptibility test with 17 antibiotics were performed by the Kirby-Bauer disk diffusion method and Minimal Inhibitory Concentrations [MICs] were determined by macrobroth dilution methodology. There is significant difference in the resistance between *Staphylococcus aureus* isolates (43(100%) and *Pseudomonas aeruginosa* isolates (32(91.4%), [P<0.05]. Resistance to ofloxacin, erythromycin, norfloxacin, tetracyclines and chloramphenicol were more common. Highly resistant isolates were selected for the drug combination. *Staphylococcus aureus* (C.I) and *Pseudomonas aeruginosa* (C.I) were used as control isolates. Erythromycin had a high MIC_{50} value against the *E coli* isolates while it had a moderate effect 47% on all the 1solates. Combination of ciprofloxacin and erythromycin was fully synergistic against one *S. pyogenes* isolate, while the same combination resulted in indifferent effect in most of the isolates. Combination therapy could be used to prevent the development of resistance and to enhanced antibacterial efficacy. Plasmid bands were seen in all the resistant isolates as revealed through agarose gel electrophoresis. The susceptibility test performed in this study suggests that nosocomial wound infections were caused by organisms that are highly resistance to antibiotics. It is imperative that hygienic conditions and surveillance initiative be focused in Hospitals in Nigeria to monitor and limit antimicrobial resistance and the spread of these nosocomial organisms, that is a major risk to human health because of its impact on morbidity, mortality and health care costs.

Keywords: Antibiotic Resistance, *Staphylococcus aureus,* disk diffusion, Antimicrobial Stewardship, surveillance

INTRODUCTION

Antimicrobial resistance (AMR) is defined using both microbiological and clinical criteria. From a microbiological perspective, a microorganism is considered resistant when it is able to grow in the presence of antimicrobial concentrations that would inhibit phylogenetically related susceptible strains, whereas clinically, resistance is demonstrated when an organism survives or continues to cause infection despite appropriate antimicrobial therapy (Frank, 2006). Antimicrobial-resistant strains occur among a wide range of bacterial pathogens, including

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staphylococci, gonococci, meningococci, pneumococci, enterococci, and Gram-negative organisms such as *Salmonella*, *Shigella*, *Escherichia*, *Klebsiella*, *Pseudomonas*, and *Mycobacterium tuberculosis* (Dawis *et al*., 2003). The emergence and dissemination of resistant bacterial pathogens have become particularly concerning in healthcare settings, where frequent antimicrobial exposure creates strong selective pressure. Methicillin-resistant *Staphylococcus aureus* (MRSA), for instance, is an increasingly important cause of healthcare-associated infections, while *Pseudomonas aeruginosa* has emerged as a major nosocomial pathogen, particularly in intensive care and burn units where antimicrobial use is often substantial (Song *et al*., 2003). The clinical significance of *P. aeruginosa* is further heightened by its remarkable genetic adaptability. Its genome contains pathogenicity islands and other mobile genetic elements capable of carrying genes associated with virulence and antimicrobial resistance, thereby facilitating the emergence and dissemination of multidrug-resistant strains (Kipnis *et al*., 2006).

Recent investigations have continued to demonstrate the importance of antimicrobial resistance among *P. aeruginosa* and other bacterial pathogens. Awari *et al.* (2024), in their assessment of pathogenic *P. aeruginosa* isolated from a hospital environment, highlighted the importance of antibiogram-based surveillance in understanding the susceptibility patterns of this important nosocomial pathogen. Similarly, Obasi *et al.* (2024) demonstrated the relevance of evaluating the activity of β-lactams and other antimicrobial agents against multidrug-resistant *P. aeruginosa*, emphasizing the continuing challenge posed by therapeutic resistance. These findings are particularly important because resistance profiles may vary considerably according to bacterial strain, antimicrobial exposure, geographical location and the environment from which the organisms are recovered.

The increasing prevalence of antimicrobial resistance also extends beyond hospital environments to community and food-associated settings. Umeoduagu *et al.* (2023a) reported on the bacteriological characteristics and antibiotic resistance patterns of bacteria isolated from fresh meat sold at Afor Oba Market, Anambra State, demonstrating the potential importance of food and community environments as reservoirs for antimicrobial-resistant organisms. Such findings reinforce concerns that resistant bacteria may circulate through interconnected human, animal, food and environmental pathways. Consequently, antimicrobial resistance should not be viewed exclusively as a hospital-based problem but as a broader public health challenge requiring integrated surveillance across different settings.

The widespread and sometimes inappropriate use of antimicrobial agents remains an important driver of resistance. In many developing countries, inappropriate prescription, self-medication, incomplete treatment courses and limited access to antimicrobial susceptibility testing may contribute to the selection and persistence of resistant organisms. The indiscriminate use of antibiotics can alter the microbial ecology of both individuals and communities, increasing the likelihood that resistant organisms will emerge and spread (Ako-Nai *et al*., 1995; Farrar, 1994). In addition, the use of antimicrobial-containing products and repeated exposure of microorganisms to suboptimal concentrations of antimicrobial agents may contribute to the selection of resistant populations.

The search for alternative and complementary approaches to antimicrobial therapy has therefore attracted increasing attention. Plant-derived products and medicinal herbs contain diverse bioactive compounds with potential antibacterial and antifungal properties and may represent useful sources of novel antimicrobial agents. Studies evaluating medicinal plants have demonstrated promising antimicrobial activities against clinically relevant bacterial pathogens. For example, Agu *et al.* (2013) reported antibacterial activity of ethanolic and petroleum ether extracts of tangerine seeds against selected bacteria, while Adindu *et al.* (2016) demonstrated phytochemical and antimicrobial properties of different parts of *Cola gigantea*. These findings suggest that naturally occurring plant-derived compounds may provide potential sources of antimicrobial substances that could complement existing therapeutic options.

The antimicrobial potential of plant-derived preparations is also relevant to the oral environment, where microorganisms such as *Streptococcus mutans* contribute to dental caries and other oral health problems. Awah *et al.* (2016) demonstrated antibacterial activity among commonly used herbal and non-herbal toothpastes against *S. mutans*, indicating that commercially available oral hygiene products may possess varying degrees of antibacterial activity. Furthermore, Awah *et al.* (2017) reported antibacterial activities of aqueous and ethanolic extracts of male

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and female *Carica papaya* leaves against selected pathogenic bacteria. Such findings provide further evidence of the potential of medicinal plants as sources of bioactive antimicrobial compounds.

The problem of antimicrobial resistance is further complicated by the possibility of inadequate or inappropriate antimicrobial activity of commonly available products. Evaluation of antimicrobial susceptibility is therefore essential for guiding treatment, monitoring resistance trends and identifying potentially effective alternative antimicrobial agents. Studies involving commercially available products have demonstrated the importance of susceptibility testing in determining their actual antimicrobial effectiveness. Umeoduagu *et al.* (2023b), for example, investigated the antimicrobial susceptibility patterns of selected isolates against commercially available toothpastes, highlighting the need to assess the efficacy of products that are widely used by the general population.

In addition to resistance among conventional bacterial pathogens, the increasing occurrence of multidrug-resistant organisms in healthcare environments represents an important threat to infection prevention and treatment. Hospital environments can serve as reservoirs for resistant organisms because of the continuous interaction between patients, healthcare workers, contaminated surfaces, medical equipment and antimicrobial agents. The isolation of pathogenic *P. aeruginosa* from hospital environments and the subsequent demonstration of varying antimicrobial susceptibility patterns further underscore the need for routine environmental surveillance and antibiogram development (Awari *et al*., 2024). Such surveillance can provide valuable information for infection-control programmes and antimicrobial stewardship initiatives.

The growing antimicrobial resistance crisis has consequently increased interest in the discovery of novel antimicrobial agents, including those derived from medicinal plants. However, the presence of phytochemicals in a plant does not necessarily guarantee clinically meaningful antimicrobial activity, making laboratory evaluation of antimicrobial efficacy essential. Phytochemical screening combined with *in vitro* antimicrobial testing can help identify plant materials with promising activity against pathogenic organisms and provide a basis for further purification and characterization of active compounds (Adindu *et al*., 2016). The investigation of plant extracts against resistant organisms may therefore contribute to the search for alternative therapeutic agents at a time when the effectiveness of several conventional antibiotics is increasingly threatened.

Overall, the continuing emergence of antimicrobial-resistant organisms, particularly multidrug-resistant Gram-negative pathogens such as *P. aeruginosa*, underscores the urgent need for effective antimicrobial surveillance, rational antibiotic use and exploration of alternative antimicrobial resources. Evidence from hospital, community, food and plant-based studies demonstrates that antimicrobial resistance is a multifaceted problem requiring approaches that integrate susceptibility testing, infection prevention, antimicrobial stewardship and the search for novel antimicrobial compounds. Against this background, investigating antimicrobial susceptibility patterns and evaluating alternative antimicrobial sources remain important strategies for addressing the growing challenge of antimicrobial resistance.

This study aimed to characterize the phenotypic and genotypic antibiotic resistance profiles of bacterial agents of nosocomial infections, evaluate the in vitro antibacterial activity of fluoroquinolone–macrolide combinations against the isolates, and investigate the occurrence and quantity of plasmid DNA among the bacterial isolates.

MATERIALS AND METHODS

Sample Collection

A total of 102 wound samples were collected from hospitalized patients between December 2008 and April 2009 at the National Orthopaedic Hospital, Enugu, and Amaku General Hospital, Awka. Samples were collected aseptically using sterile swab sticks and transported without delay to the Microbiology Laboratory, Nnamdi Azikiwe University, Awka, for microbiological analysis. Nosocomial infection was defined as an infection that developed 48 h or more after hospital admission. Where more than one bacterial isolate was recovered from a patient, only one representative isolate was selected for inclusion in the study.

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Isolation and Preservation of Bacterial Isolates

The wound specimens were inoculated onto nutrient agar, cetrimide agar, blood agar, and MacConkey agar using the streak-plate technique as previously described by Cheesbrough (2002). The inoculated plates were incubated aerobically at 37°C for 24–48 h. Following incubation, colonies exhibiting distinct morphological characteristics were selected and subcultured repeatedly to obtain pure cultures. Pure isolates were maintained on agar slants and stored appropriately for subsequent identification and antimicrobial susceptibility investigations.

Identification and Characterization of Bacterial Isolates

The bacterial isolates were identified using standard microbiological procedures based on colonial morphology, cellular morphology, Gram-staining characteristics, spore formation, and a range of conventional biochemical tests. The biochemical characterization included oxidase, catalase, indole, methyl red, urease, citrate utilization, Voges–Proskauer, nitrate reduction, motility, and carbohydrate fermentation tests involving glucose, maltose, sucrose, mannitol, fructose, lactose, and xylose. Identification of isolates to the genus level was performed using the schemes described in *Bergey's Manual of Determinative Bacteriology*.

Gram Staining

Gram staining was performed on smears prepared from 24-h-old bacterial cultures. A thin smear was made on a clean, grease-free glass slide, allowed to air-dry, and heat-fixed by passing the slide briefly through a Bunsen burner flame. The smear was flooded with 0.5% crystal violet for 60 s, rinsed briefly with water, and treated with Lugol's iodine for approximately 30 s. After rinsing, the preparation was decolorized with acetone–alcohol and immediately washed with water. The smear was subsequently counterstained with safranin for 30 s, rinsed, and allowed to air-dry. The stained preparations were examined microscopically using the ×100 oil-immersion objective. Gram-positive organisms retained the crystal violet stain and appeared violet, whereas Gram-negative organisms appeared pink to red following counterstaining.

Motility Test

Bacterial motility was determined using the hanging-drop technique. Young cultures were prepared by inoculating the isolates into peptone water and incubating them at 37°C for 24 h. A drop of the resulting culture was placed on a clean coverslip, and the coverslip was carefully inverted over a concave slide to obtain a hanging drop. The preparation was examined under the ×40 objective of a light microscope. Motility was inferred from the characteristic directional movement of bacterial cells, as distinguished from Brownian motion.

Oxidase Test

Oxidase activity was determined using filter paper moistened with a 1% solution of tetramethyl-p-phenylenediamine dihydrochloride. A portion of bacterial growth was transferred onto the reagent-moistened filter paper using a sterile wire loop. Development of a purple colour within 10 s was interpreted as a positive oxidase reaction.

Catalase Test

Catalase activity was determined by emulsifying a portion of each bacterial isolate in a drop of 3% hydrogen peroxide on a clean glass slide. Immediate production of visible gas bubbles, resulting from the breakdown of hydrogen peroxide into water and oxygen, was recorded as a positive catalase reaction.

Carbohydrate Fermentation Test

Carbohydrate fermentation was assessed using peptone water containing the appropriate carbohydrate, bromothymol purple as the indicator, and an inverted Durham tube for detection of gas production. The prepared

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media were sterilized at 115°C for 10 min, allowed to cool, and inoculated with the test organisms. The cultures were incubated at 37°C for 24 h. Fermentation was indicated by a colour change of the medium from light purple to yellow, signifying acid production, while the accumulation of gas in the Durham tube indicated gas production.

Indole Test

Indole production was determined by inoculating young bacterial cultures into peptone water and incubating them at 37°C for 48 h. Following incubation, Kovac's reagent was added to the cultures. The development of a dark-red colour in the surface layer was interpreted as a positive indole reaction.

Methyl Red Test

The methyl red test was performed using buffered glucose-peptone broth. The test medium was inoculated with the bacterial isolates and incubated for 48 h. Subsequently, five drops of methyl red reagent were added to 5 mL of the culture. Development of an immediate bright-red colour was interpreted as a positive methyl red reaction, indicating stable acid production from glucose fermentation.

Urease Test

Urease production was determined by inoculating the bacterial isolates into urea-containing medium and incubating the cultures for 24 h at 30°C. The urea base was prepared separately and supplemented with sterile 20% (w/v) urea solution after cooling. A change in the colour of the medium to pink was considered indicative of urease production.

Citrate Utilization Test

Citrate utilization was determined using Simmon's citrate agar. A loopful of each bacterial culture was inoculated onto the medium and incubated for 24 h at room temperature. Utilization of citrate as the sole carbon source was indicated by growth accompanied by a colour change of the medium from green to blue.

Voges–Proskauer Test

Voges–Proskauer testing was performed using 48-h-old broth cultures. Approximately 1 mL of culture was treated with α-naphthol and 0.2 mL of 40% potassium hydroxide, with the preparation shaken after the addition of each reagent. A small quantity of creatine was added to enhance the reaction. Development of a pink to red colour was interpreted as a positive Voges–Proskauer reaction, indicating acetoin production.

Nitrate Reduction Test

Nitrate reduction was assessed using cultures grown in indole nitrate broth for 48 h. Following incubation, the appropriate volumes of sulfanilic acid reagent and α-naphthol reagent were added sequentially. Development of an orange-red colour within 10 min was interpreted as evidence of nitrate reduction to nitrite.

Spore Staining

Endospore formation was determined using the Schaeffer–Fulton staining method. A thin smear of each bacterial isolate was prepared on a clean glass slide, air-dried, and heat-fixed. The smear was stained with malachite green while being gently steamed to facilitate penetration of the stain into the endospores. The preparation was subsequently rinsed with water and counterstained with safranin for approximately 30 s. After washing and drying, the stained preparations were examined microscopically under the ×100 oil-immersion objective. Endospores appeared green against the red or pink vegetative cells and were recorded as positive for spore formation.

Coagulase Test

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Coagulase production was determined using the slide coagulase method. A 24-h-old bacterial culture was emulsified on a clean, grease-free glass slide, after which a loopful of human plasma was added and mixed thoroughly with the bacterial suspension. Visible agglutination or clumping occurring within 10 s was interpreted as a positive coagulase reaction.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic susceptibility tests were performed with the standardized disk- diffusion method on Mueller-Hinton agar, according to the Kirby - method and following the guidelines of the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 2002) Catridges of antimicrobial - containing disc were obtained from POLY-TES MED Laboratories, Agbani, Road, Enugu, Nigeria, stored between 4 and 8°C and allowed to come to room temperature prior to use. Staphylococcus aureus (C.I), and *Pseudomonas aeruginosa* (C.I) were used as an internal control. The antibiotic disks used were Ofloxacin (5µg) Erythromycin (10µg), Gentamicin (10µg), Cephalexin (30µg). Cotrimoxazole (50µg) Cloxacillin (30µg), Floxapen (30µg), Augumentin (30µg), Nitrofurantoin (100µg), Amoxycillin (20µg), Tetracycline (50µg). Norfloxacin (10µg), Chloramphenicol (10µg), Cefuroxime (30µg) and Ampicillin (10µg).

Procedure

A sterile wire loop was used to touch 5 discrete colonies of the isolates grown for 24hrs at 37°C in a Mueller-Hinton agar and inoculated into 4mls of nutrient broth and were incubated for 4-6hrs, then diluted to suspensions with turbidities equivalent to that of 0.5 McFarland standard to yield a final inoculum of 10'cfuml. A sterile cotton tipped swab was dipped into each of the tubes with the standard inoculum of the isolates and excess fluid were removed by pressing and rotating the swab against the side of the tube above the level of the suspension and then the swab was used to streak uniformly into the plates of Mueller-Hinton agar. After, this antimicrobial discs with known concentrations of antimicrobials were placed on the inoculated plates of Mucller - Hinton agar using sterile forceps, then within 30minutes of applying the discs, the plates were inverted and incubated aerobically at 37°C for l6 to l8hours. Zones of inhibition were measured and interpreted using NCCLS (National Committee for Clinical Laboratory Standard).

Determination of the Minimal Inhibitory Concentrations (MICS) and Minimal Bactericidal Concentration (MBC)

MICs were determined in triplicate by macro broth dilution technique (NCCLS, 2004). Different concentrations of the antimicrobials: Ciprofloxacin (Micro labs Limited, Hosur, India). Erythromycin (Medreich plc, Surrey, England) were obtained by two fold serial dilution. The final concentrations tested were ciprofloxacin 0.125 - 1024mg/L and Erythromycin 0.125 - 1024 mg/L. A control tube containıng broth without antimicrobial agent was used for each isolate tested. Inoculum was prepared by the direct colony suspension method as follows; three to five identical colonies were selected from young cultures and suspended in 4mls of nutrient broth, then incubated for 24hrs at 37°C. The suspension was further diluted to meet the 0.5 McFarland standard to obtain a final inoculum of 10^5cfu/ml). The standard test inocula were inoculated into the different tubes of two-fold dilusion containing the different concentrations of the antimicrobials in a nutrient broth. The tubes were incubated at 37°C for 18-24hrs. The MIC was defined as the lowest concentration of antibiotic completely inhibiting visible growth after 18 - 24hours of incubation at 37°C. *Staphylococcus aureus* (C.I) and *Pseudomonas aeruginosa* (C.I) were used as control organisms for each antıbiotic tested.

MBC determination was by aspırating 0.Iml of the culture medium from each tube (in the macro broth MIC assay) showing no apparent growth and sub-culturing it on fresh Mueller Hinton agar (MHA), then they were incubated at 37°C for 24hours. The MBC was read as the least concentration showing no visible growth on the MHA subculture.

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Drug combination testing

Highly resistant 10 isolates were selected for the synergy study. They include four isolates of *Staphylococcus aureus*, two *Pseudomonas aeruginosa*, two isolates of *Escherichia coli* and two isolates of *Streptococcus pyogenes.* The synergy study were performed in a 96- well microtitre plates containing two antimicrobial agents (Ciprofloxacin and Erythromycin) in 2- fold dilutions. For the combination, containing ciprofloxacin plus erythromycin in nutrient broth (Lab M, UK) were prepared in a checkerboard fashion. Suspensions with turbidities equivalent to that of a 0.5 McFarland standard were prepared to yield a final inoculum of 10^5cfu/ ml. The range of concentrations used in the combination studies was determined according to the previously determined MIC of each antibiotic for each specific isolates. After overnight incubation, MICs were read. The fractional inhibitory concentration (FIC) for each agent was calculated according to the following formula.

FIC of drug A (FIC_A) = $\frac{\text{MIC of drug A in combination}}{\text{MIC of drug A alone}}$

FIC of drug B (FIC_B)

$FIC_B = \frac{\text{MIC of drug B in combination}}{\text{MIC of drug B alone}}$

Drug A = Ciprofloxacin

Drug B = Erythromycin

Fractional inhibitory concentration index (FICI) of the combination was calculated according to the following formula.

$FICI = FIC_A + FICs$

$$FICI = \left(\frac{\text{MIC of drug A in combination}}{\text{MIC of drug A alone}} \right) + \left(\frac{\text{MIC of drug B in combination}}{\text{MIC of drug B alone}} \right)$$

The results were interpreted as follows FICI $\leq$ 0.5, Synergistic; 0.5 < FICI <l, partially synergistic; FICI = 1, additive, 1 > FICI $\leq$ 4, indifferent; and FICI >4, antagonistic

Isolation and Quantification of Plasmid DNA

Plasmid DNA was isolated from the bacterial isolates using the alkaline lysis DNA miniprep method described by Lech and Brent (1987), with minor modifications. Briefly, 1.5 mL of an overnight bacterial broth culture was transferred into a microcentrifuge tube and centrifuged at 14,000 rpm to pellet the bacterial cells. The supernatant was carefully decanted, leaving approximately 50–100 µL above the cell pellet, which was subsequently vortexed at high speed to achieve complete resuspension.

The resuspended cells were lysed by adding 300 µL of TENS solution, consisting of 25 mM Tris, 10 mM EDTA, 0.1 M NaOH, and 0.5% sodium dodecyl sulfate (SDS). The contents were gently mixed by inverting the tube five times until a viscous mixture was obtained. Thereafter, 150 µL of 3.0 M sodium acetate (pH 5.2) was added, and the mixture was vortexed thoroughly to ensure uniform mixing. The lysate was then centrifuged at 14,000 rpm for 5 min to precipitate cellular debris and chromosomal DNA.

Following centrifugation, the resulting supernatant containing plasmid DNA was carefully transferred into a fresh sterile microcentrifuge tube and mixed with 900 µL of ice-cold absolute ethanol to precipitate the plasmid DNA. The mixture was centrifuged at 14,000 rpm for 10 min, after which a whitish pellet, presumed to contain the precipitated plasmid DNA, was observed. The supernatant was carefully discarded, and the pellet was washed twice with 1 mL of 70% ethanol. The pellet was subsequently air-dried and resuspended in 20–40 µL of Tris-EDTA (TE) buffer for further analysis and downstream applications.

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Electrophoresis

Electrophoresis of the plasmid DNA was carried out on a 0.8% agarose gel in a 1X concentration of Tris-Borate EDTA (TBE) buffer. The agarose gel was prepared by boiling 0.8g of agarose powder in 100mls of IX TBE buffer. After boilıng, the solution was allowed to cool and 10µL of ethidium bromide was added to the cooled agarose solution. This was poured into a casting tray with a comb placed across its rim to form wells. The gel was allowed to set for 30minutes and the comb was removed. Plasmid DNA samples were then loaded into the wells after mixing with 2µL of bromophenol blue. A DNA molecular weight marker (HIND II1 digest of lambda, DNA) was also loaded into one of the wells. The gel was then electrophoressed in horizontal tank at a constant voltage.

RESULTS

The identification of the bacterial isolates associated with nosocomial infection are shown in tables l and 2. Table 3 shows the antibiotic resistance among Gram positive isolates The antibiotic resistance among Gram negative isolates are shown in table 4 Tables 5 and 6 show the phenotypic antibiotic resistance patterns of some Gram positive and Gram negative isolates respectively. Table 7 showed resistance of *S.aureus* and *P.aeroginosa* isolates to non -β-lactam antibiotics. There is a significant difference in the susceptibility of *S. aureus and P. aeroginosa* isolates to the non β -lactam antibiotics at $P < 0.05$. The minimum inhibitory concentrations of ciprofoxacin and erythromycin for the isolates are shown in table 8. The drug combination study for the two antimicrobial tested against the selected resistant isolates is illustrated in table 9. Electrophoresis of the plasmid DNA from selected antimicrobial resistant isolates are shown in figures 2 and 3

Table 1: Characterization of the Gram Positive isolates

Probable identity of the isolates	Physiological reaction						Acid from		
	Gram Stain	Motility	Catalase	Growth on 10% NaCL	Voges-Proskaauer	β – haemolysis	Lactose	Mannitol	Ribose
Staphylococcus aureus	+	-	+	+	+	-	+	+	+
Streptococcus pyogens	+	-	-	-	-	+	+	-	-

Key:

+ : Positive

- : Negative

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Table 2: Characterization of the Gram Negative isolates

Probable identity of the isolates	Physiological reaction: Gram Stain	Motility	Urea	Voges-Proskauer	Oxidase	Catalase	Nitrate	Growth at 42^{0}C	Citrate	Indole	Methyl red	Acid from: Mannitol	Maltose	Glucose
Pseudomonas auruginosa	-	+	Nd	Nd	+	+	+	+	Nd	Nd	Nd	+	-	+
Escherichia coli	-	+/-	-	-	-	+	+	-	-	+	+	+	Nd	-

Key:
+/-: Positive or negative
+: Positive
-: negative
Nd: Not determined

ANTIBIOTIC RESISTANT PROFILES OF THE ISOLATES

The antibiotic resistance profiles of the isolates are shown in tables 3 and 4. Of the *S.aureus* isolates, 42(97.7%) were resistant to ofloxacin, erythromycin and augumentin. Resistance to ciprofloxacin was 100% for the *S. aureus* and *S. Pyogenes*. Resistance to gentamicin was higher in *S. aureus* than in *S pyogenes* (Table 3) This may be due to the occurrence of methicillin- resistance *Staphylococcus aureus*. The rate was very high with 35(100%) of *P. aeruginosa* and 10(100%) of *E. coli* to at least three tested antibiotics (Table 4). The susceptibility was very low with all the antibiotics tested in all the isolates.

Synergy was detected with *S. pyogenes* - 26, partial synergy and indifference were detected in two *S. aureus* isolates and one *P. aeruginosa* isolate respectively. In all the genera, there is an indifferent isolate. Antagonism is seen in only one *E coli* isolate. The synergistic interaction seen in the *S. pyogenes* - 26 shows that the combination of ciprofloxacin and erythromnycin enhanced antimicrobial activity on this isolate. The antagonistic effect showed that the drug combination has no effect on the resistance of this isolate. Erythromycin and ciprofloxacin showed synergy or partial synergy against I(10%) and 3(30%) of the tested isolates respectively. Indifference is seen in 5(50%) of the tested isolates as shown in table 9.

Table 3: Antibiotic resistance among Gram positive isolates

	Staphylococcus aureus			***Streptococcus pyogenes***		
Antibiotics	**Number of Isolates**			**Number of Isolates**		
	S	**I or R**	**% Resistance**	**S**	**I or R**	**% Resistance**
Ofloxacin	1	42	97.7	0	8	100

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Erythromycin	1	42	97.7	1	7	87.5
Ciprofloxacin	0	43	100	0	8	100
Clindamycin	4	39	90.7	2	6	75
Gentamicin	8	35	81.4	2	6	75
Cephalexin	0	43	100	0	8	100
Cotrimoxazole	0	43	100	0	8	100
Cloxacillin	0	43	100	0	8	100
Floxapen	0	43	100	0	8	100
Augumentin	1	42	97.7	0	8	100

Key:
S: Susceptible
R: Resistant

Table 4: Antibiotic resistance among Gram negative isolates

	Pseudomonas aeruginosa			***Escherichia Coli***		
Antibiotics	**Number of Isolates**			**Number of Isolates**		
	S	**I or R**	**% Resistance**	**S**	**I or R**	**% Resistance**
Ofloxacin	5	30	85.7	2	8	80.0
Ciprofloxacin	3	32	91.4	1	9	90.0
Gentamicin	3	32	91.4	0	10	100
Nitrofurantoin	1	34	97	0	10	100
Tetracycline	3	32	91.4	2	8	80.0
Norfloxacin	0	35	100	2	8	80.0
Amoxycillin	0	35	100	1	9	90.0
Chloramphenicol	0	35	100	1	9	90.0
Cefluroxime	0	35	100	0	10	100

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Ampicillin	0	35	100	0	10	100

Key;
S: Susceptible
I: Intermediate
R: Resistant

Table 5: Phenotypic antibiotic resistance patterns of some Gram positive isolates

Organism – number on Antibiotics

Sample	CIP	CD	GN	CX	CO	AU	FX	OF
S.aureus – 49	R	R	R	R	R	R	R	R
S. aureus – 25	R	R	R	R	R	R	R	R
S. aureus – 37	R	R	R	R	R	R	R	R
S. aureus – 39	I	R	S	R	R	R	R	R
S. pyogenes	I	S	S	R	R	R	R	R

Key:
CIP: Ciprofloxacin, CD: Clindamycin, GN: Gentamicin, CX: Cephalexin,
CO: Cotrimoxazole, AU: Augumentin, FX: Floxapen, OF: Oflaxacin
R: Resistant, I: Intermediate, S. Susceptible

Table 6: Phenotypic antibiotic resistance patterns of some Gram negative isolates

Organism – number on Antibiotics

Sample	CIP	GN	OF	AM	CF	AX	NB	TE
P.aeruginosa – 27	R	I	R	R	R	R	R	R
P.aeruginosa – 47	I	S	R	R	R	R	R	S
P.aeruginosa – 52	R	R	R	R	R	R	R	I
Escherichia coli – 72	I	R	S	R	R	S	R	R

Key:
CIP: Ciprofloxacin, GN: Gentamicin, OF: Oflaxacin, AM: Ampicillin,
CF: Cefuroxime, AX: Amoxycillin, NB: Norfloxacin, TE: Tetracycline
R: Resistant, I: Intermediate, R. Resistant

Table 7: Resistance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates to non β – lactam antibiotics.

Non-β-lactam antibiotics	**N (%) n = 78**	***S.aureus* n = 43**	***P.aeruginosa* *n = 35***
Gentamicin	67 (85.9%)	35 (81.4%)	32 (91.4%)
Ciprofloxacin	75 (96.2%)	43 (100%)	32 (91.4%)
Ofloxacin	72 (94.7%)	42 (97.7%)	30 (85.7%)
Clindamycin	39 (50%)	39 (90.7%)	(ND)
Erythromycin	42 (53.8%)	42 (97.7%)	(ND)
Tetracycline	32 (41%)	(ND)	32 (91.4%)
Nitrofurantoin	34 (43.6%)	(ND)	34 (97.1%)

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Key:
ND: not determined

Minimum Inhibitory Concentrations of ciprofloxacin and erythromycin for the isolates

MIC_{50} and MIC_{90} values represent the concentrations at which 50% and 90%of the 1solates respectively were inhibited, The ciprofloxacin MICs for *S.aureus* ranged from 0.5 to 16mg/L. In *P aeruginosa* isolates, the erythromycin MICs ranges from 2 -> 256mg/L. In E coli, the MIC_{50} and MIC_{90} to ciprofloxacin is 4mg/L. This shows that *E coli* is very susceptible to ciprofloxacin at low concentrations (Table 8). *Streptococcus pyogenes* is susceptible to ciprofloxacin with MIC range of 0.25 8mg/L and (MIC_{50} = 8mg/L). *S. aureus* isolates were very susceptible to ciprofloxacin with 50% and 90% of the isolates inhibited at 1mg/L.

Table 8: Minimum Inhibitory Concentrations for the organisms tested

		MIC (mg/L)		
Organism (no of isolates)	**Antibiotics**	**Range**	**MIC_{50}**	**MIC_{90}**
Staphylococcus aureus (4)	Ciprofloxacin	0.5 – 16	1	1
	Erythromycin	8 – 128	32	128
Pseudomonas aeruginosa (35)	Ciprofloxacin	2 – 32	4	32
	Erythromycin	2-> 256	4	32
Escherichia coli (10)	Ciprofloxacin	2 – 4	4	4
	Erythromycin	64 – 256	64	128
Streptococcus pyogenes (8)	Ciprofloxacin	0.25 – 8	8	8
	Erythromycin	0.5 -> 16	2	8

Table 9: Drug combination test results for Ciprofloxacin and Erythromycin against the selected resistant isolates

Organism – no of isolate	**Antibiotic combination**	**FICI**	**Interpretation**
Staphylococcus aureus – 25	Ciprofloxacin	1.5	Indifferent
	+		
Staphylococcus aureus – 74	Erythromycin	0.62	Partially Synergistic
Staphylococcus aureus – 73	"	0.52	Partially Synergistic
Staphylococcus aureus – 32	"	4	Indifferent
Streptococcus pyogenes – 26	"	0.25	Synergistic
Streptococcus Pyogenes – 19	"	2.5	Indifferent
Pseudomonas aeruginosa – 44	"	0.56	Partially Synergistic
Pseudomonas aeruginosa – 71	"	2	Indifferent
Escherichia coli - 24	"	1.25	Indifferent
Escherichia coli -51	"	4.25	Antagonism

FICI: Fractional Inhabitory Concentration Index

PLASMID PROFILE OF DNA OF ISOLATES

In the electrophoresis of the plasmid DNA, plasmid band was observed in all the isolates (fig3 and 4). In fig 3, all the isolates showed a DNA band of about 22.5kbp, but *P.aeruginosa* - 61 had another plasmid band of 9.6kbp while

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in fig 4, the plasmid DNA band is about 19.5kbp and *Streptococcus pyogenes* - 26 has two plasmid band of 2.8kbp and 2.0kbp. This shows that the antibiotic resistance in these isolates could be plasmid-mediated

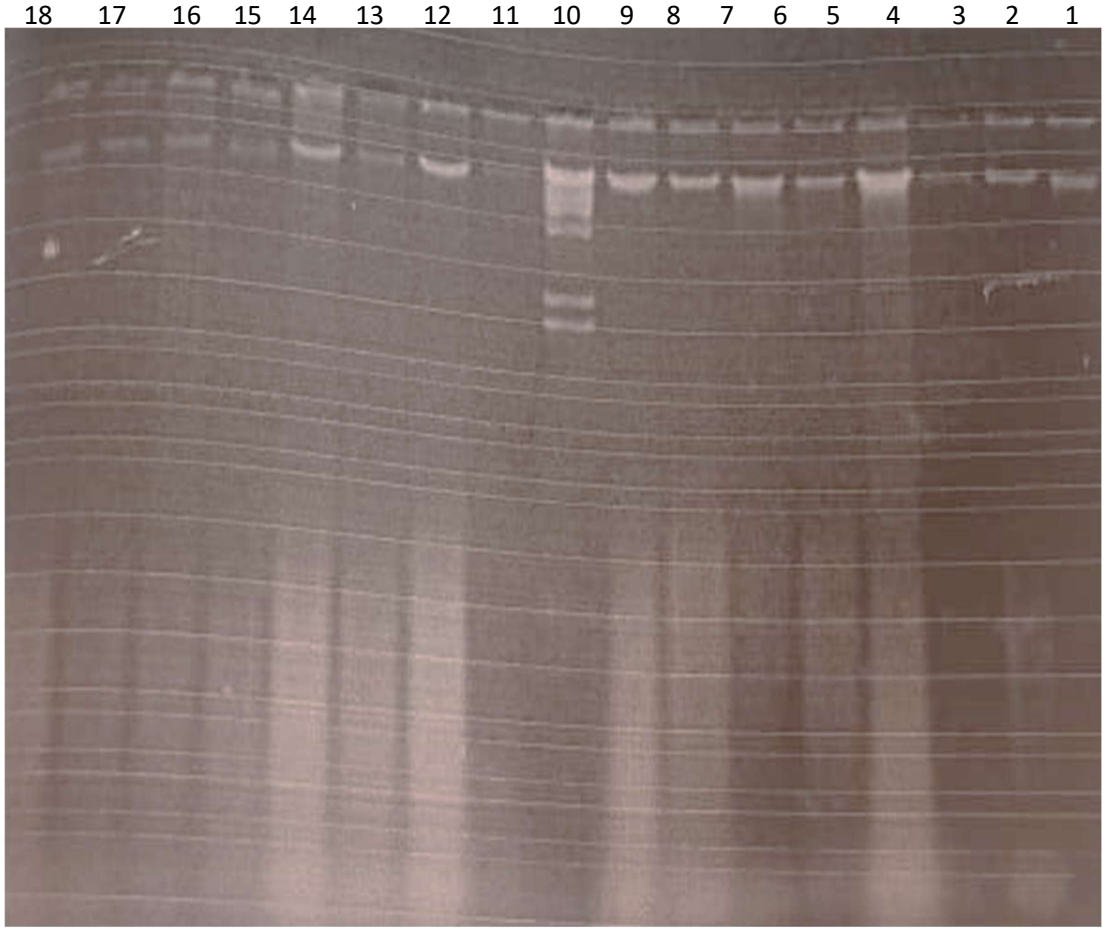

Figure 2: Plasmid profile of DNA of Selected antimicrobial resistant isolates I

Lane 1: *Pseudomonas aeruginosa – 13*	Lane 10: *Pseudomonas aeruginosa – 47*
Lane 2: *Pseudomonas aeruginosa – 18*	Lane 11: *Pseudomonas aeruginosa – 48*
Lane 3: *Staphylococcus aureus*- 32	Lane 12: *Pseudomonas aeruginosa – 59*
Lane 4: *Pseudomonas aeruginosa – 41*	Lane 13: *Pseudomonas aeruginosa – 61*
Lane 5: Staphylococcus aureus - 42	Lane 14: *Pseudomonas aeruginosa – 64*
Lane 6: Streptococcus pyogenes - 43	Lane 15: *Pseudomonas aeruginosa – 71*
Lane 7: *Pseudomonas aeruginosa – 44*	Lane 16: *Pseudomonas aeruginosa – 75*
Lane 8: *Pseudomonas aeruginosa – 45*	Lane 17: *Pseudomonas aeruginosa – 78*
Lane 9: Marker (HND III digest of lambda DNA	Lane 18: *Escherichia coli - 14*

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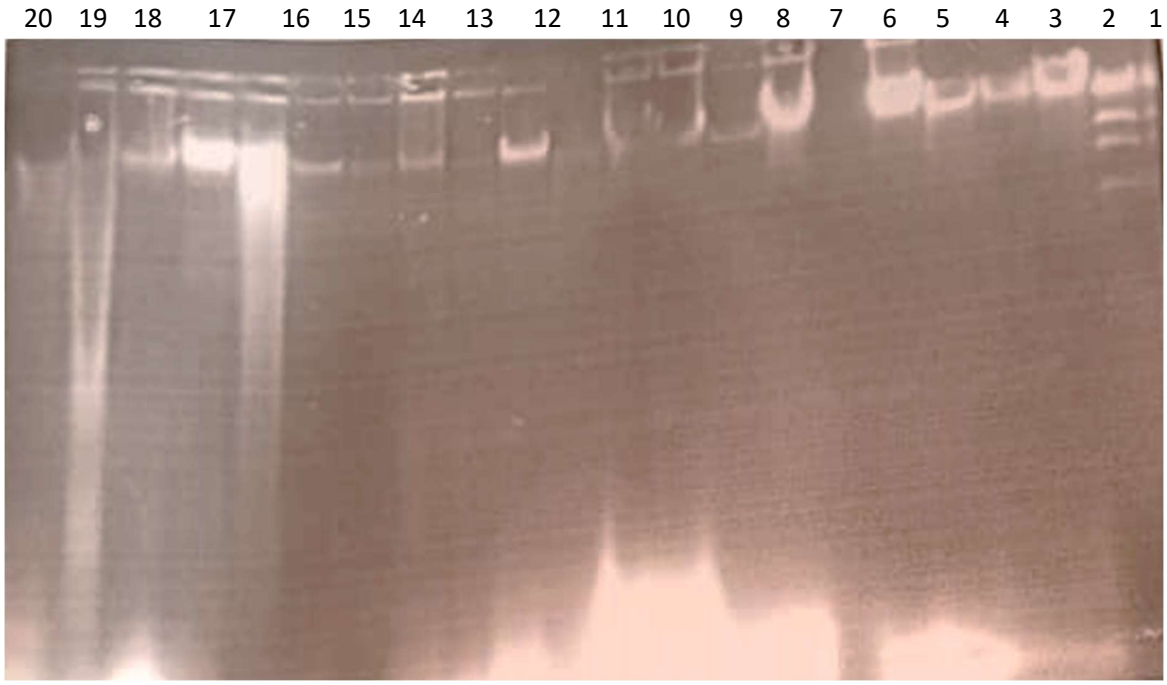

Figure 3: Plasmid Profile of DNA of Selected antimicrobial resistant isolates II

Lane 1: Marker (HND III digest of lambda DNA	Lane 11: *Streptococcus Pyogenes – 19*
Lane 2: *Escherichia - 24*	Lane 12: *Staphylococcus aureus – 25*
Lane 3: *Staphylococcus aureus*- 29	Lane 13: *Staphylococcus aureus - 30*
Lane 4: *Escherichia coli – 33*	Lane 14. *Staphylococcus aureus – 37*
Lane 5: Pseudomonas aeruginoasa - 36	Lane 15: *Staphylococcus aureus – 40*
Lane 6: *Staphylococcus aureus* – 38	Lane 16: *Staphylococcus aureus – 74*
Lane 7: *Staphylococcus aureus – 49*	Lane 17: *Staphylococcus aureus – 73*
Lane 8: *Escherichia coli – 51* Lane 9: *Pseudomonas aeruginoasa - 52*	Lane 18: *Staphylococcus aureus – 87* Lane 19: *Staphylococcus aureus - 26*
Lane 10: *Pseudomonas aeruginoasa* - 59	Lane 20: *Staphylococcus aureus - 12*

DISCUSSION

The results of this study showed that most nosocomial (wound) infections were caused by organisms *(Staphlococcus aureus*, *Pseudomonas aeruginosa, Eshcerichia coli and Streptococcus pyogenes*) that were highly resistant to antibiotics and this correlated with the report of Diekema *et al (*2001). The results also provided important data on antimicrobial resistance and the drug-drug Interactions which helps to evaluate that the combination of ciprofloxacin and erythromycin is synergistic on *Streptococcus pvogenes* - 26. The drug combination has enhanced activity and also prevented the emergence of resistance on this isolate.

Potential synergistic interactions between antimicrobials have been investigated for years. Severe nosocomial infections caused by *P. aeruginosa, S. aureus* and *E coli*, particularly in deblitated patients, requires aggressive treatment usually involving at least two antimicrobial agents. In this study, partial synergy and indifference were detected in two *S. aureus* isolates and one *Pseudomonas aeruginosa* isolate. Antagonism was seen in only one isolate of E. coli. The drug combination showed indifference with 50% of the isolates tested. Woottan *et al* (1995) concluded that chequerboards should only be used with adequate controls and replications.

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In this statement, it was found useful of assessing antibiotic interactions although possibly too labour intensive for routine use in a diagnostic Laboratory. It has found extensive use in the study of antibiotic combinations for treatment of multi-resistant organisms (Owens et al.,1997).

Erythromycin, due to its suppression on RNA synthesis, inhibits new cycles of chromosome replication, so causing DNA gyrase to be inactive in low concentrations There are clinical reports on use of ciprofloxacin for treatment of infections caused by methicillin- resistant *Staphylococcus aureus* (MRSA) (Maple et al., 1987). In this study, it was found that 100% and 91.4% of the *S. aureus* and *P. aeruginosa* isolates, respectively, were resistant to, ciprofloxacin(Table 7), In other studies conducted in African hospitals, resistance to methicillin varied from 21 to 63% in South Africa and from 21 to 31% in Cameroon, Nigeria, Kenya, Ivory-Coast and Ethiopia (Kesah et al., 2003).

Rates of resistance to antibiotics tested were in the range of 75% to l00%. However, ciprofloxacin was less active against *E coli* isolates while it was more active on *P. aeruginosa* isolates. 50% and 90% of *S. aureus* isolates were inhibited at Img/L. This correlates positively with the finding of Thomsom et al. (1999).

The significance of the above in vitro findings must be confirmed in the clinical setting, but properly randomized and controlled clinical trials may not be feasible to perform. This study showed greater efforts to simplify the analytical approach of antimicrobial combinations, based on dilutions, that are needed to promote greater appreciation of rational antimicrobial combination therapeutic regimens. This guidance may lessen the selective pressures existing in many medical facilities with constantly increasing resistant microbial populations challenging the rapid recovery of patients.

Plasmid -DNA bands were observed from the selected resistant isolates as revealed through the gel electrophoresis. This showed that the antibiotic resistant genes could be coded by plasmid- DNA and not chromosomal DNA. It is interestingly that these resistant plasmıd DNA can be transferred between the bacteria causing the nosocomial infections. This means that the resistance will be very high in hospital admitted patients because of patient- to patient contact. It is because of this reason that it is imperative that surveillance initiatives be focused on both hospital and community in order to monitor and limit the spread of these nosoconmial infections, also further extensive genetic studies are necessary to detect other resistance mechanisms involved in nosocomial infections.

CONCLUSION

The result revealed that *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and Streptococcus pyogenes were isolates obtained from nosocomal infections studied. The susceptibility studies showed that these isolates were highly resistant to antibiotics. It was also observed that the resistant genes of these isolates are coded by plasmid - DNA as revealed through the gel electrophoresis. The synergistic effect observed with the drug combination provides a practical advantage that drug combination enhances antibacterial action and also prevent the emergence of resistance. This study acknowledges the fact that tradıtional approaches to antibiotic prescribing, that is, treating susceptible infections with narrow-spectrum antimicrobials, fail to address the frequency with which resistance is encountered, and, thus expose patients to the risk of ineffective therapy

In the course of this study, it was found that the understandıng of local patterns of nosocomial infection, local susceptibility and antibiogram information will help physicians to develop strategies that can effectively improve treatment in hospitalized patients with serious nosocomial infections.

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