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COMPARATIVE ASSAY OF CIPROFLOXACIN AND DOXYCYCLINE BRANDS MARKETED IN PORT HARCOURT METROPOLIS NIGERIA AGAINST *Staphylococcus aureus* CLINICAL ISOLATE

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ABSTRACT

Staphylococcus aureus is among the most prominent causes of bacterial infections and because of its unique nature, it can quickly respond to new antibiotic with the development of efficient mechanisms to neutralize them. Undoubtedly, this has left fewer effective drugs to treat these often-life-threatening infections. The aim of this study was to evaluate the potency of ciprofloxacin and doxycycline brands marketed within Port Harcourt Metropolis against *Staphylococcus aureus* clinical isolate obtained from the Department of Microbiology University of Port Harcourt Teaching Hospital, Nigeria. The minimum inhibitory, sub inhibitory and bactericidal concentrations (MIC, MBC) of the test antibiotics were determined using standard methods. The MIC range for ciprofloxacin was 0.01953125 - 0.065104166 mg/ml, ½ × MIC, 0.009765625 - 0.032552066 mg/ml, 2 × MIC, 0.0390625 - 0.130208333 mg/ml and MBC 0.625-2.5 mg/ml. The MIC results for doxycycline ranged from 0.000325521 - 0.026041666 mg/ml, ½ × MIC 0.00016276 - 0.013020833 mg/ml, 2 × MIC 0.000651042 - 0.05208333 and MBC 0.078125 - 0.3125 mg/ml. In this study, sensitivity and effectiveness of doxycycline is higher than that of ciprofloxacin. The different brands showed variation in their sensitivity and effectiveness against the test microorganism. This disparity may be attributed to several factors including pharmacological variability between drug batches or between generic and originator drugs, incorrect drug quantity and presence of impurities, acquisition of resistance through mutations in some of their genes when they are exposed to an antibiotic. A concerted effort is required to ensure that only medicines of acceptable quality reach the end user.

KEYWORDS: *Staphylococcus aureus*, minimum inhibitory concentration, minimum bactericidal concentration, ciprofloxacin, doxycycline, brands, sensitivity.

INTRODUCTION

The emergence of *Staphylococcus aureus* strains that are highly resistant to antimicrobials has recently become a major public health concern.[1] Interestingly, the organism is a widespread commensal bacterium and pathogen.[2,3] It has an extraordinary repertoire of virulence factors that allows it to survive extreme conditions within the human host.[4] Not less than 50% of individuals are intermittently or permanently colonized with *S. aureus* thus, there is relatively high potential for infections.[5,6] The primary natural reservoir of *S. aureus* in human beings is the squamous epithelium of the anterior nares.[3] Three major pattern of colonization has been reported and includes, persistent carriers (roughly 20% of the general population), intermittent carriers (30%), and non-carriers (50%).[7] Despite constant improvement in patient care, *S. aureus* infections remain associated with considerable morbidity and mortality, both in hospitals and in the community.[8] Infections may occur as a result of inoculation of *S. aureus* into an open wound. More commonly, affected are the skin, mucosal surfaces, upper airway, viral infection damages to the mucosal linings which predisposes the host to *S. aureus* pneumonia, and classically presents a week after onset of influenza infection.[4] Initial exposure of *S. aureus* to host tissues beyond the mucosal surface or skin is thought to trigger upregulation of virulence genes.[9] For the host, resident phagocytes and epithelial cells in the skin or mucosal tissue respond to either bacterial products or tissue injury by activation of the immune system. *S. aureus* peptidoglycan and lipoprotein are sensed by host pattern recognition molecules,[10,11] hyaluronan breakdown products[12] and endogenous toll like receptor ligands, ribonucleic acid (RNA), Deoxyribonucleic acid (DNA), high mobility group box 1 (HMGB1) released by necrotic tissues,[13] during infection further augment pro-inflammatory signaling leading to local immune cell activation, and neutrophil and macrophage recruitment. *S. aureus* has been generally recognized to survive well both inside and outside of host cells. In the extracellular

milieu, *S. aureus* must overcome opsonization by complement and antibodies, which directly or indirectly leads to killing of *S. aureus* or uptake by phagocytes through Fc or complement receptors.[4] *S. aureus* avoids opsonophagocytosis by expressing on its surface a capsule, clumping factor A, protein A, and a number of complement inhibitors, all of which inactivate or prevent host opsonins from binding or targeting the bacterium for destruction.[14, 15] Transmission of *S. aureus* infections is mostly by direct or indirect contact with a person who has a discharging wound or clinical infection of the respiratory or urinary tract or who is colonized with the organism. Hands of healthcare personnel can harbour Methicillin-Resistant *Staphylococcus aureus* (MRSA), this can likely be a mode of transmission between patients and staff including contaminated surfaces and medical equipment.[2,3]

Previous report shows that *S. aureus* is a major human pathogen that causes a wide range of clinical infections.[2] It is a leading cause of scalded skin syndrome, a relatively rare, toxin-mediated disorder with clinical features varying from superficial localized blisters to generalized exfoliation.[16,17] Bone infections (osteomyelitis), in children, sudden onset of fever and bone tenderness or a limp; pain may be throbbing and severe; however, presentation in neonates can be subtle.[18] Septic arthritis Septic arthritis typically presents as a hot, swollen, tender joint or joints with a reduced range of movement.[19] Endocarditis initially presents as fever and malaise; peripheral emboli may be present; may involve healthy valves.[20] Toxic shock syndrome presents as fever, diffuse macular erythema, and hypotension, with involvement of three or more organ systems with capacity of rapidly progressing in previously healthy individuals.[21] Skin and soft tissue infections (SSTIs) ranging from mild infections, such as pyoderma, cellulitis, impetigo, folliculitis, carbuncles to serious life-threatening infections, such as necrotizing fasciitis[22-26] pleuropulmonary, and device-related infections.[2] Infections due to *S. aureus* are treated with antibiotics, however such choices depend on the severity of the infection, clinical presentation and the results of susceptibility testing.[27] Linezolid, daptomycin, telavancin and ceftaroline are some of the drugs that have received regulatory approval in the last decade for the treatment of infections caused by drug-resistant Gram-positive pathogens.[28] In this study, ciprofloxacin and doxycycline were selected because they are among the most commonly prescribed and dispensed antibiotics in this locality for the treatment of infections caused by *S. aureus*, with SSTIs inclusive. Ciprofloxacin, a fluoroquinolone, is a potent, broad-spectrum antibacterial agent. The fluoroquinolone enters the bacterium by passive diffusion through water-filled protein channels (porins) in the outer membrane. Once inside the cell, they inhibit the replication of bacterial DNA by interfering with the action of DNA gyrase (topoisomerase II) and topoisomerase IV during bacterial growth and reproduction.[29] Topoisomerases are enzymes that change the configuration or topology of DNA by nicking, pass-through, and resealing mechanism.[30] It is active against a wide range of gram-negative organisms, including *Pseudomonas aeruginosa*, and it has some activity against gram-positive agents, including *Staphylococcus aureus* and many strains of *Streptococcus pneumoniae*. In addition, the drug is highly active against many organisms responsible for causing atypical pneumonia, including *Mycoplasma*, *Chlamydia*, and *Legionella*. Ciprofloxacin penetrates the cellular membrane, making the drug ideal for attacking intracellular pathogens like *Salmonella*. Ciprofloxacin has excellent bioavailability and is not highly protein bound. Clearance of ciprofloxacin is by both renal and non-renal pathways and approximately, two third of metabolites are excreted in the urine and 15% in feces.[29] For doxycycline, multiple reports support it use in patients with suspected or confirmed cutaneous community- acquired Methicillin- resistant *Staphylococcus aureus* (CA-MRSA) infection.[31] Doxycycline is a synthetic tetracycline derivative, consist of four fused rings with a system of conjugated double bonds.[30] Substitution on these rings are responsible for variation in the drugs' individual pharmacy kinetics, which cause small differences in their clinical efficacy.[30] Entry of these agents into susceptible organisms is mediated both by passive diffusion and by an energy-dependent transport protein mechanism unique to the bacterial inner cytoplasmic membrane. The drug binds reversibly to the 30S subunit of the bacterial ribosome, thereby blocking access of the amino acyl-tRNA to the mRNA-ribosome complex at the receptor site. By this mechanism, bacterial protein synthesis is inhibited.[30] Widespread resistance to the tetracycline limits their clinical use. The most commonly encountered, naturally occurring resistance (R) factor confers an inability of the organism to accumulate the drug, thus producing resistance. This is accomplished by Mg^{2+} -dependent, active efflux of the drug, mediated by the plasmid-encoded resistance protein, TetA. Any organism resistant to one tetracycline is resistant to all.[30] Recognition of the extent, depth and severity of infection is paramount if appropriate and timely therapeutic intervention is to be achieved.[32] This study therefore assesses the potency of multiple brands of two antibiotics (ciprofloxacin and doxycycline) against clinical isolates of *S. aureus*. Studies like these are important to check the effectiveness of antimicrobial agents used for treatment to avoid sub-therapeutic effect or ineffectiveness that could lead to resistance.

MATERIALS AND METHODS

Culture Media

The media used in this study includes, Nutrient Agar, Nutrient broth, Muller Hinton broth (Titan Biotech Ltd India). The media were constituted according to manufacturer's specification. Sterilization was by autoclaving at 121°C for 15 min and maintained in molten form until ready for use.[33-35]

Test organism

A clinical isolate of *Staphylococcus aureus* was obtained from the department of Microbiology, University of Port Harcourt Teaching Hospital (UPTH) Rivers State, Nigeria. Stock culture was maintained on slopes of modified nutrient agar at 4^{o}C and sub-cultured routinely.

Test Antibiotics

Five brands of ciprofloxacin and doxycycline were obtained from approved Pharmacy outlets within Port Harcourt Metropolis, South-south region, Nigeria and coded as A, B, C, D and E. All the antibiotic samples were inspected to ensure that manufacturer's information, batch numbers, manufacturing and expiry dates, National Agency for Food and Drug Administration and Control (NAFDAC) Registration Number were provided. The samples were transported to pharmaceutical microbiology laboratory, University of Port Harcourt for evaluation.

Preparation of Test Antibiotics

Stock concentration of ciprofloxacin was prepared by dissolving 500 mg of the drug in 25ml of sterile water to produce a stock of 20 mg/ml while that of doxycycline was prepared by dissolving 100 mg of the drug in 20 ml of sterile water to obtain stock of 5 mg/ml.

Standardization of test organism

The McFarland standards are commonly used in antibiotic susceptibility test to standardize the approximate number of bacteria in a liquid suspension or broth culture of the bacterial cell by comparing the turbidity of the cultured test suspension with that of the McFarland standard.[36] A loopful of the purified culture was picked using a flamed and cooled sterile wireloop, introduced into a sterile peptone water in a universal bottle. The bottle was shaken, and the turbidity compared with that of the McFarland standard. The adjusted bacterial suspension was used within 15-20 minutes.[36]

Determination of Minimum Inhibitory Concentration (MIC)

The stock concentration was diluted serially to produce several dilutions in decreasing order of concentration. To the first tube containing 5 ml of sterile double strength nutrient broth, 5 ml of the test agent was added, the resultant mixture was mixed thoroughly, 5 ml was then transferred from the first tube to the second containing 5ml of single strength nutrient broth and was mixed thoroughly, after which, 5ml volume was transferred from the second to third and the process repeated sequentially up to last tube. Thereafter, 0.1 ml of the test culture was inoculated into each of the dilutions and incubated at 37^{o}C for 24 hours. The procedure was adapted for the remaining brands of ciprofloxacin and doxycycline. Microbial growth was examined visually for the presence of turbidity in the incubated tubes. The lowest concentration of the agent that inhibits growth of test organism was taken as the MIC.[36]

Determination of 50% and 200% MIC

Half (fifty percent) or two times (two hundred percent) the concentrations of the various MIC values were prepared from the stock solution as previously described.[36] 0.1 ml of the test organism was inoculated into each of the dilutions and incubated at 37^{o}C for 24 hours. Microbial growth was examined visually for the presence of turbidity in the incubated tubes.

Determination of Minimum Bactericidal Concentration (MBC)

Following the MICs determination, a volume of the reaction mixture was transferred from tubes showing no visible growth to corresponding containers of fresh nutrient broth, acting as the cell recovery medium in triplicates. The newly inoculated tubes were then incubated at 37°C for 48 hours.[36] The minimal concentration of the antimicrobial agent that produces total cell death was taken as the MBC.[36] The procedure was carried out for all brands of both doxycycline and ciprofloxacin and result were recorded accordingly.

Statistical analysis

The data obtained on activities of the test antibiotics against the test isolates were analyzed using One - way analysis of variance (ANOVA). All the data were computed as means ± standard deviation using GraphPad Prism 7. Probability value of less than or equal to 0.05 is considered to be statistically significant.

RESULTS AND DISCUSSION

Minimum Inhibitory Concentration/ Minimum Bactericidal Concentration

In this study, five brands of ciprofloxacin and doxycycline antibiotics were examined for their resistance and sensitivity to clinical isolates of *Staphylococcus aureus*. The results of minimum inhibitory concentrations (Fig. 1 – 6) and minimum bactericidal concentrations (Fig. 7 - 8) against the test organism are presented below.

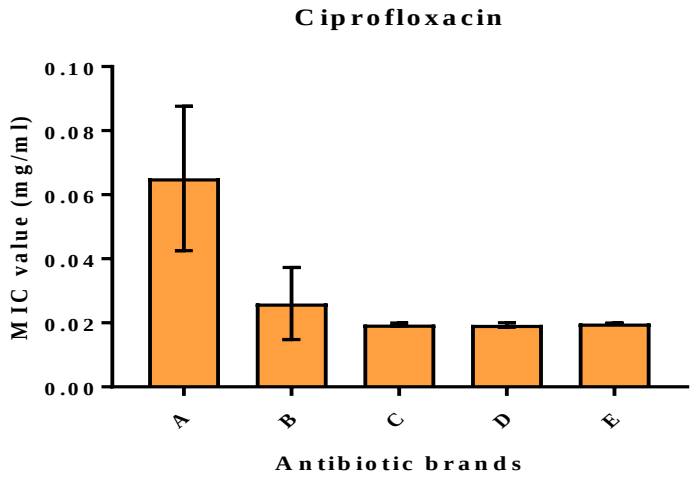

Fig. 1: Column chart showing inhibitory concentration produced by Ciprofloxacin brands against test organism. P value = <0.002. Data shown are from three independent cultures with error bars indicating standard deviations.

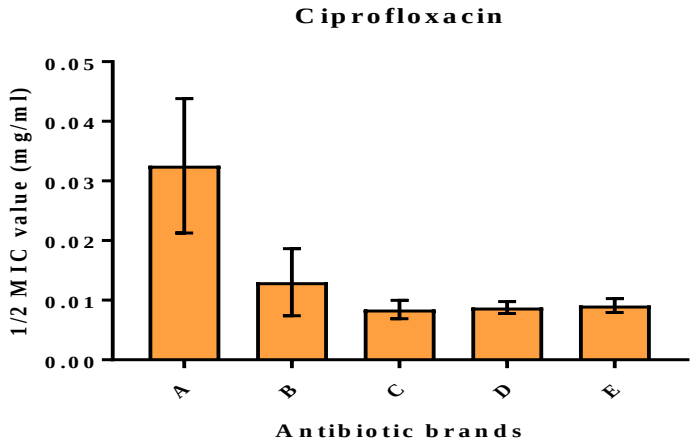

Fig. 2: Column chart showing inhibitory concentration produced by Ciprofloxacin brands against test organism. P value = <0.001. Data shown are from three independent cultures with error bars indicating standard deviations.

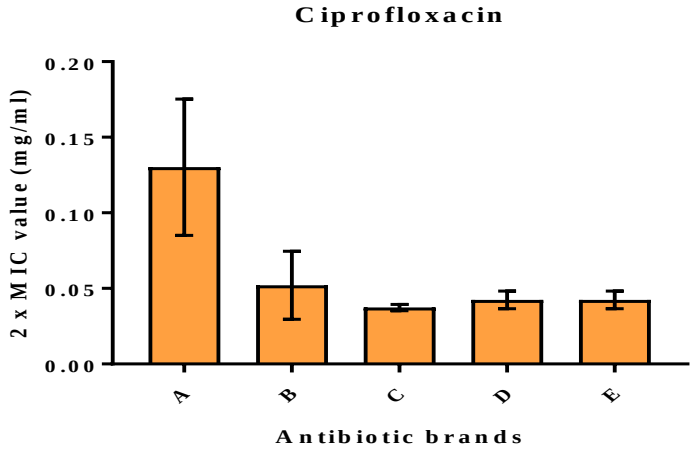

Fig. 3: Column chart showing inhibitory concentration produced by Ciprofloxacin brands against test organism. P value = <0.002. Data shown are from three independent cultures with error bars indicating standard deviations.

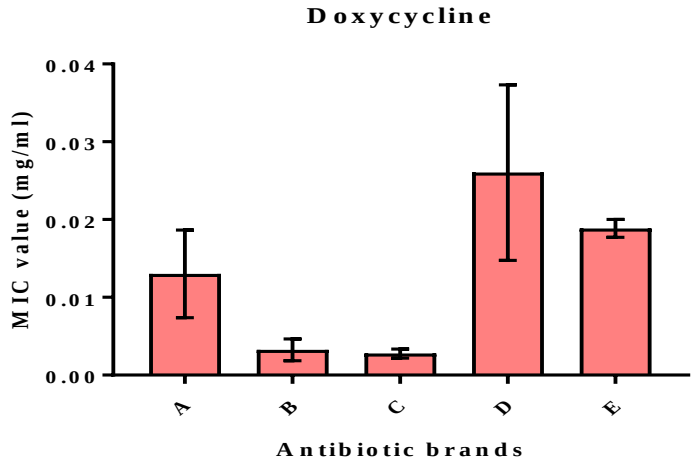

Fig. 4: Column chart showing inhibitory concentration produced by Doxycycline brands against test organism. P value = <0.002. Data shown are from three independent cultures with error bars indicating standard deviations.

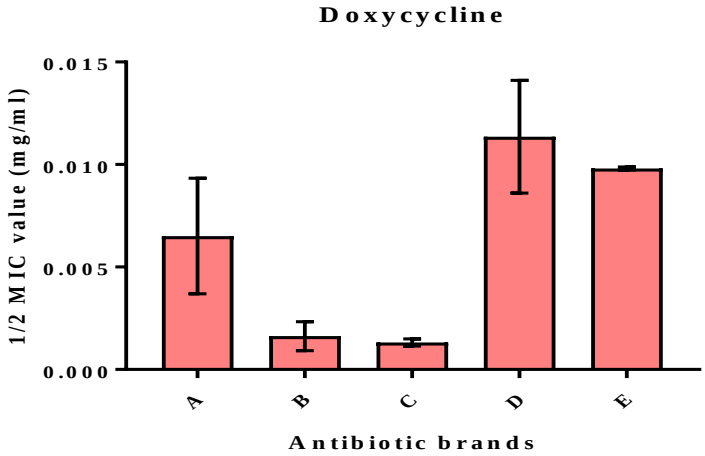

Fig. 5: Column chart showing inhibitory concentration produced by Doxycycline brands against test organism. P value = <0.0001. Data shown are from three independent cultures with error bars indicating standard deviations.

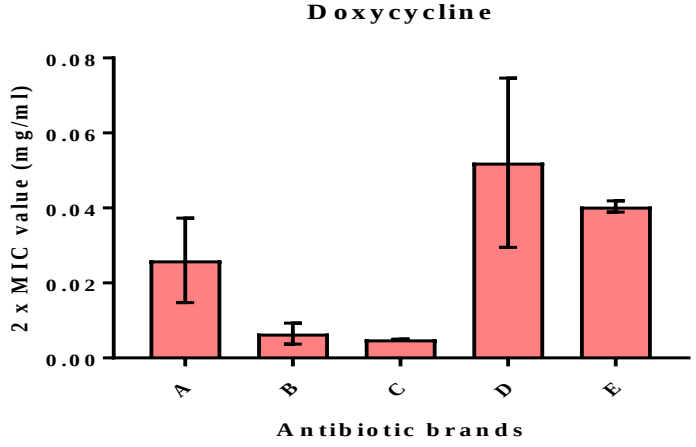

Fig. 6: Column chart showing inhibitory concentration produced by Doxycycline brands against test organism. P value = <0.001. Data shown are from three independent cultures with error bars indicating standard deviations.

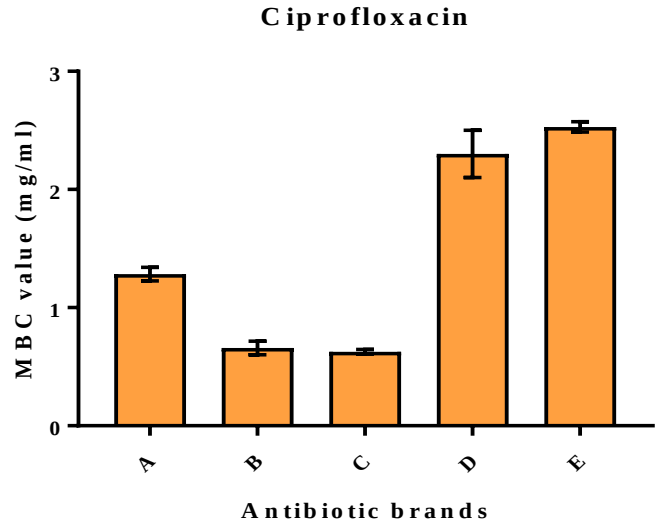

Fig. 7: Column chart showing inhibitory concentration produced by Ciprofloxacin brands against test organism. P value = <0.0001. Data shown are from three independent cultures with error bars indicating standard deviations.

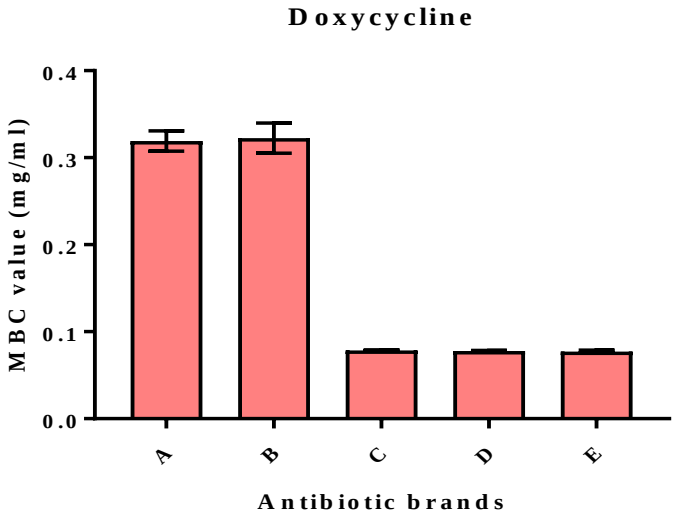

Fig. 8: Column chart showing inhibitory concentration produced by Doxycycline brands against test organism. P value = <0.0001. Data shown are from three independent cultures with error bars indicating standard deviations.

From the results, the least sensitive brand is brand A with average MIC value of 0.065104066 mg/ml. Brand B and C tends to be more effective against the clinical isolates owning to the fact they had the least concentration that are able to kill the microorganism completely (average MBC, 0.625 mg/ml). This is followed by brand A with MBC value of 1.25 mg/ml. The least efficacious with regard to the clinical isolate is samples D and E with average MBC value of 2.5 mg/ml. Comparing the sensitivity and effectiveness of the doxycycline used, it was observed that brand B is more sensitive in inhibiting the growth of the microorganism. This is due to the fact that at a very minute concentration of 0.000325521 mg/ml, it was able to inhibit the growth of the organism. Followed by brand C (average MIC of 0.01953125 mg/ml). Comparing the effectiveness of the different brands of doxycycline used it could be inferred that samples C-E are more effective. This is due to the fact that they had the least concentration of MBC, 0.078125 mg/ml followed by samples A and B average MBC value, 0.3125 mg/ml. The reasons for variation in MIC and MBC values for the different brands of antibiotic may include substandard formulation, deliberate fraudulent practices,[37] prior exposure of isolate to sub inhibitory concentration[38] and inability to completely release the active ingredient from other excipient used in the manufacture of the dosage form.[39] acquisition of resistance through mutations in some of their genes when they are exposed to an antibiotic.[40] Previous report indicates poor-quality medicines can reach the market

through substandard production of legitimate drugs due to inadequate quality-control processes during manufacture, as well as by deliberate fraudulent practices. Undoubtedly, this present a serious public health problem, and could have a significant impact on emerging economies and developing countries.[37] Comparing the drugs, it was observed that doxycycline is more effective against *S. aureus* than ciprofloxacin. Consequently, a lesser concentration of doxycycline is required to inhibit and completely kill *S. aureus*, as oppose to ciprofloxacin.

Bacteria resistance among clinical isolates has been developed due to excessive and irrational use of antibiotics. Treatment failure of antibiotic therapy resulted from emergence of such resistance. In developing countries mostly, broad-spectrum antibiotic are prescribed without analyzing reports of antibiotic susceptibility test.[38] Even though ciprofloxacin appears to be safe and effective for a wide variety of clinical infections the emergence of *S. aureus* resistance to ciprofloxacin is of great concern. Two principal mechanisms of fluoroquinolone resistance have also been reported. The first involves point mutation in the grlA/grlB and gyrA/gyrB genes, which encodes the subunits of topoisomerase lV and DNA gyrase respectively.[41] The second mechanism involves efflux of fluoroquinolones by the membrane-associated protein NorA. This efflux pump actively transports fluoroquinolones and several other structurally unrelated compounds out of the bacterial.[41] Resistance of *S. aureus* to tetracycline has also been reported and is achieved by two potential mechanisms, active reflux and ribosomal protection. Active reflux is mediated by plasmid-located on tetK and tetL genes, whereas, chromosomes located on tetM or tetO genes mediate ribosomal protection.[42] Doxycycline and minocycline are two tetracycline analogues that are reportedly more effective against Gram-positive bacteria than were earlier tetracycline derivatives. Both antibiotics have been reported to be more effective against *S. aureus*.[43] Doxycycline is a good choice of drug for skin and structure infections due to *S. aureus*, particularly CA-MRSA but has poor anti-streptococcal activity and has side effect of photosensitivity.[31] From the studies carried out, it showed that both drugs, doxycycline and ciprofloxacin are effective in management of infections caused by *S. aureus.* There is variability among the different brands of antibiotic used. In case of the ciprofloxacin brands used, brands C-E showed similar and greatest sensitivity against the clinical isolates, *S. aureus* with average MIC values of 0.01953125 mg/ml each, followed by brand B with average MIC value of 0.026041666 mg/ml.

CONCLUSION

This study assesses the potency of multiple brands of ciprofloxacin and doxycycline against clinical isolates of *S. aureus* by determining the inhibitory and bactericidal concentrations using standard methods. It was observed that doxycycline is more effective against *S. aureus* than ciprofloxacin. The different brands also showed variation in their sensitivity and effectiveness against the test microorganism. This variation may be due to substandard drug formulation, deliberate fraudulent practices or the acquisition of resistance. There is no doubt that there is the need for an antibiotic surveillance program development and implementation by relevant agencies as a way of ensuring that only medicines of acceptable quality reach the patient.

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