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# Susceptibility Pattern of *Staphylococcus aureus* and *Pseudomonas spp*. Isolates from Chronic Wounds to Some Locally Produced Antibiotics

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## Abstract

High demand of antibiotics has resulted in the production of substandard and counterfeit antibiotics which impact badly on chronic wounds and is a concern to the health-care providers due to limited treatment options. This study identifies the susceptibility pattern of *Staphylococcus aureus* and *Pseudomonas* spp isolates from chronic wounds to some locally produced antibiotics in the treatment of wounds at two facilities in Ghana. Two hundred and sixty (260) wound swabs were collected from chronic wound patients visiting the SPH and NGH from December 2016 to June 2017. Bacterial isolates were characterized using standard microbiological techniques. Seven locally produced antibiotics were tested against *Staphylococcus aureus* and *Pseudomonas* spp. Antimicrobial susceptibility and sensitivity testing was done using Kirby- Baur disk diffusion method and the CLSI guidelines. Phenotypic identification of MRSA and ESBL was also done. A total of 211 bacterial isolates were recovered showing an isolation rate of 81.2%. Staphylococcus aureus (30.8%) was the predominate organism isolated, followed by *Pseudomonas aeruginosa* (24.9%). The results identified 21 (28.8%) out of 73 *staphylococcus aureus* isolates as MRSA and 35% of the *Pseudomonas* spp. to be ESBL. 6 out of the 7 locally produced antibiotics tested showed biological activity similar to the standard commercial controls. Overall, our findings indicate that even though most of the isolates were resistance to β-lactam antibiotics such as amoxicillin and flucloxacillin, the susceptibility profile and mean zones of inhibition of most local antibiotics tested were statistically comparable ($p > 0.05$) to standard controls.

***Key Words:*** *Antimicrobials; Susceptibility; Antibiotics; Chronic; β-lactam*

**Abbreviations:** SPH: St. Patrick's Hospital; NGH: Nkenkaasu Government Hospital; CLSI: Clinical and Laboratory Standards Institute; MRSA: Methicillin Resistance *Staphylococcus aureus*; MSSA: Methicillin Sensitive *Staphylococcus aureus* MSSA; ESBL: Extended spectrum beta-lactamase

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## Introduction

Wounds infection by bacteria and resistance to common antibiotics are the common post-surgical and medical challenges and therefore the fight against microbial infections has been boosted in recent years by local pharmaceutical companies through the manufacturing of several types of antibiotics. However, this widespread manufacturing and use of antibiotics, together with the length of time over which they have been available have led to major problems of resistant to wound infecting bacteria [Sani., *et al*. 2012]. The high demand of antibiotics has also resulted in the production of substandard and counterfeit antibiotics into the global supply chain, and therefore fueling resistance which subsequently increase the complications and costs of treatment [Anguzu and Olila 2007]. Though locally manufactured antibiotics are now readily available, relatively inexpensive, and widely prescribed to patients, some researchers believe some of these drugs are of very poor quality [Newton., *et al*. 2010]. World Health Organisation (WHO) estimates that more than 40% of all drugs in circulation in Africa are counterfeit whilst the Institute of Research against Counterfeit Medicines (IRACM) in 2013 found that over 500 million doses of poor quality drugs worth about 275 million US dollars seized in 23 African countries included Nigeria and Ghana [Digital Journal, 2013]. Certainly, the development of antibiotics has lengthened our lifespan, it is very obvious that poor manufacturing processes, excessive and inappropriate used of these drugs may be causing serious long-term life-threatening effects, that we are only now becoming fully aware of [Egbo, 2013].

It is reported that, multidrug-resistant Gram negative bacterial strains such as *Pseudomonas aeruginosa*, and Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with infections due to extensive mis prescription and inadequate dose regimen of antibiotics over the last decade [Sani., *et al*. 2012]. [Mama., *et al*. 2014] also reported that among the prevalent organisms that have been associated with wound infection, *Staphylococcus aureus* account for 20-40% whilst *Pseudomonas aeruginosa* account for 5-15% of the nosocomial infection. The study therefore seeks to identify Staphylococcus aureus and *Pseudomonas* spp present in infected wounds and examine their resistance profile to some common locally produced antibiotics used in therapy and compare their susceptibility patterns to standard commercial controls. This will help determine the potency of local brand of antibiotics in the fight against bacterial infections.

## Materials and Methods

### Sample Collection and Culture

Two hundred and thirty-seven wound swab samples were collected aseptically from 260 patients attending the St. Patrick's Hospital and Nkenkaasu Government Hospital from December 2016 to June 2017 for wound dressing services and analysed. The swabs were inoculated on Blood agar, MacConkey agar, and Chocolate agar (Oxoid). All the bacteria isolates were identified using morphological, microscopy and biochemical standard procedures.

### Antibiotics Susceptibility Testing

Antibiotic susceptibilities of the recovered bacterial isolates were determined using the method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2016). Seven locally-produced and commonly prescribed antibiotics (ciprofloxacin 5µg, cefuroxime 30 µg, clindamycin 2 µg, flucloxacillin 5 µg, amoxicillin 20 µg, amoxiclav 20/10 µg and metronidazole 25 µg) were bought from reputable pharmaceuticals shops and standard commercial controls (Oxoid, ThermoFisher, Scientific, USA) were tested for their biological activity on isolated *Staphylococcus aureus* and *Pseudomonas* spp. These antimicrobials were selected based on their availability and prescription frequency in the study area. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 Barium sulphate solution. The test organism was uniformly seeded over the Kirby-Bauer disc diffusion technique according to criteria set by CLSI (2016) and then incubated at 37°C for 16–18 hours. The zones of inhibition were measured and the isolates classified as sensitive (S), intermediate (I), and resistant (R) according to the CLSI tables and guidelines.

**Detection of MRSA and ESBL**

Detection of MRSA isolates was done using 30 µg cefoxitin discs on Mueller Hinton agar plate. Bacterial broth suspension of 0.5 McFarland standard was prepared and used. 16-18 hours after incubation at 37°C, the zones of inhibition were measured to the nearest millimetre using a ruler and interpreted according to CLSI (2016) criteria; bacteria with zone size ≥ 22 mm was classified as MSSA and ≤ 21 mm as MRSA. For ESBL detection, 30 µg of ceftazidime discs was used as an indicator to identify resistance or diminished susceptibility isolates likely to be harbouring ESBLs. One confirmation test discs contained ceftazidime alone and the other in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10µg ). An increase of ≥ 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer.

**Data analysis**

The quantitative variables were expressed as means ± SD while qualitative variables were presented as frequencies. Data was analyzed using statistical package for social science (SPSS) version 17. p-value of < 0.05 was considered to indicate statistically significant differences. The result was presented using tables.

**Ethics**

Ethical clearance was obtained from the committee on Human Research, Publication and Ethics of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Written informed consent was obtained from all study participants.

## Results and Discussion

**Culture results**

Bacterial wound infection is a serious problem in the hospital and the treatment of wound infections remain a significant concern for surgeons. The problem has been magnified due to the unrestrained and rapidly spreading resistance to the available array of antimicrobial agents.

Out of the 260 chronic wound swabs taken, 211 (81.2%) showed growth whereas 18.8% were negative for growth. Of a total of 237 bacteria isolates obtained, 85 (35.9%) were Gram positive and 152 (64.1%) were Gram negative which are in agreement to several earlier studies. Staphylococcus aureus 73 (30.8%) was the predominate organism isolated, followed by *Pseudomonas* spp. 59 (24.9%). Even though our results contradict that of Zhang., *et al*. (2014), previous reports on wound infection from different parts of the world have also shown that *S. aureus* and *E. coli* were the most frequent isolates [Bhatt., *et al*. 2006] [Mulu., *et al*. 2012]. The high prevalence of *S. aureus* infection may be that, it is an endogenous source of infection or may be due to contamination from the environment, for example, from surgical instrument.

**Antibiotic susceptibility and resistance profile of Staphylococcus aureus (MRSA and MSSA)**

In the determination of the susceptibility, out of the 73 *Staphylococcus aureus* isolates, 21 (28.8%) were detected to be Methicillin Resistance *Staphylococcus aureus* (MRSA) by cefoxitin disc diffusion method. This correlates with an earlier study by Newman., *et al*. (2015), which found the resistance rate of MRSA to be 22%. On the contrary, studies elsewhere showed 50.2% [Eksi., *et al*. 2011] and 75% [Udobi., *et al*. 2013] in Turkey and Nigeria respectively. S. aureus and MRSA are major cause of soft tissue infections in hospitalized patients whilst other reports have also implicated Pseudomonas, *Staphylococcus, Streptococcus*, Klebsiella, and *E. coli* in wound infections [Misic., *et al*. 2014].

All the MRSA (100%) were resistant to the locally-produced amoxicillin (Table 1), which may be attributed to very poor quality, extensive use of these drugs, differences in antibiotic administration, infection prevention measures, population size, and the method of testing as well as the study design [Dibah., *et al*. 2014]. Peacock and Paterson, (2015) also reported of a mecA gene, which encodes

a penicillin-binding protein (PBP2a) with lower affinity for β-lactams and therefore antibiotics targeting PBPs are no longer effective against the bacterial cell. This low activity was followed by amoxiclav (86%) and flucloxacillin (81%); but, only 10% showed resistance to ciprofloxacin. (Table 1). In all cases, the resistance rate of the standard controls and their respective locally-produced antibiotics, as well as the mean zones of inhibition were not significantly different ($p > 0.05$) except cefuroxime. Studies by Egyir., *et al*. (2015) reported high MRSA resistance to this class of antibiotics. However, 85% and 62% of the MRSA strains tested were susceptible to ciprofloxacin and clindamycin respectively (Table 1). This finding is in agreement with some previous study which proposed that ciprofloxacin and clindamycin could be used as an alternative therapy to vancomycin for MRSA infection [Pai., *et al*. 2010].

| Code | Antibiotics | S (%) | X (mm) | I (%) | R (%) | T test value | P value |
|---|---|---|---|---|---|---|---|
| $S^c$-CIP | Ciprofloxacin (5µg) | 90 | 27 ± 2.5 | 0 | 10 | 0.105 | 0.917 |
| L-CIP | Ciprofloxacin (5µg) | 85 | 27 ± 2.7 | 5 | 10 | | |
| $S^c$-CXM | Cefuroxime (30µg) | 52 | 24 ± 1.8 | 0 | 48 | 4.776 | 0.000 |
| L-CXM | Cefuroxime (30µg) | 0 | - | 33 | 77 | | |
| $S^c$-CLI | Clindamycin (2µg) | 67 | 27 ± 3.0 | 0 | 33 | 0.881 | 0.386 |
| L-CLI | Clindamycin (2µg) | 62 | 28 ± 2.9 | 5 | 33 | | |
| $S^c$-FLU | Flucloxacillin (5µg) | 24 | 22 ± 1.2 | 0 | 76 | 0.287 | 0.776 |
| L-FLU | Flucloxacillin (5µg) | 19 | 21 ± 0.5 | 0 | 81 | | |
| $S^c$-AMC | Amoxicillin (20µg) | 0 | - | 5 | 95 | -0.134 | 0.894 |
| L-AMC | Amoxicillin (20µg) | 0 | - | 0 | 100 | | |
| $S^c$-AMOC | Amoxiclav(20/10µg) | 14 | 24 ± 0.6 | 0 | 86 | -0.103 | 0.918 |
| L-AMOC | Amoxiclav(20/10µg) | 14 | 23 ± 0.6 | 0 | 86 | | |
| $S^c$-MTZ | Metronidazole(25µg) | N/T | N/T | N/T | N/T | N/D | N/D |
| L-MTZ | Metronidazole(25µg) | 0 | - | 0 | 100 | | |

Key: S = Sensitive; I = Intermediate; R = Resistant; x = mean sensitive zone of inhibition; n = Total organisms tested; L = local brand; $S^c$ = standard commercial control; CIP – Ciprofloxacin; CXM – Cefuroxime; CLI – Clindamycin; FLU – Flucloxacillin; AMC – Amoxicillin; AMOC – Amoxiclav; MTZ – Metronidazole; N/D = Not done; N/T = Not tested.

***Table 1:*** *Antimicrobial susceptibility patterns of MRSA against different standard controls and local brands antibiotics by the disc diffusion method and their mean susceptible zones of inhibition (n = 21).*

Contrary to the MRSA results, methicillin sensitive Staphylococcus aureus (MSSA) were susceptible to all the standard control and the local brands (Table 2). 100% of the MSSA showed susceptibility to ciprofloxacin with mean zone of inhibitions of 30±3.7 mm and 29 ± 3.5 mm for the standard commercial control and local brand respectively. Nonetheless, our results are at variance with report from Zabielinski et al. (2013) who observed increased antibiotic resistance among MSSA to ciprofloxacin and clindamycin. Cefuroxime, flucloxacillin, amoxiclav, and amoxicillin showed 92%, 50%, 48% and 40% susceptibility respectively. This compares favorably with findings from other African countries (Dilnessa and Bitew, (2016); Onwubiko and Sadiq, (2011). Amoxicillin showed the least susceptibility of 40%. However, apart from cefuroxime, there were no statistical significant differences ($p > 0.05$) between the susceptible mean zones of inhibition of the local brand of antibiotics and their respective standard controls (Table 2).

| Code | Antibiotics | S (%) | X (mm) | I (%) | R (%) | T test value | P value |
|---|---|---|---|---|---|---|---|
| $S^c$-CIP | Ciprofloxacin (5µg) | 100 | 29 ± 3.3 | 0 | 0 | -1.852 | 0.067 |
| L-CIP | Ciprofloxacin (5µg) | 100 | 30 ± 3.9 | 0 | 0 | | |
| $S^c$-CXM | Cefuroxime (30µg) | 63 | 24 ± 1.4 | 2 | 35 | 5.337 | 0.000 |
| L-CXM | Cefuroxime (30µg) | 0 | - | 46 | 54 | | |
| $S^c$-CLI | Clindamycin (2µg) | 92 | 28 ± 3.5 | 2 | 6 | -0.720 | 0.473 |
| L-CLI | Clindamycin (2µg) | 92 | 28 ± 3.5 | 2 | 6 | | |
| $S^c$-FLU | Flucloxacillin (5µg) | 56 | 24 ± 1.8 | 10 | 35 | 0.538 | 0.592 |
| L-FLU | Flucloxacillin (5µg) | 50 | 24 ± 1.6 | 8 | 42 | | |
| $S^c$-AMC | Amoxicillin (20µg) | 42 | 22 ± 1.3 | 0 | 58 | -0.112 | 0.911 |
| L-AMC | Amoxicillin (20µg) | 40 | 21 ± 3.0 | 0 | 60 | | |
| $S^c$-AMOC | Amoxiclav(20/10µg) | 46 | 24 ± 2.1 | 4 | 40 | -0.125 | 0.901 |
| L-AMOC | Amoxiclav(20/10µg) | 48 | 24 ± 1.7 | 2 | 50 | | |
| $S^c$-MTZ | Metronidazole(25µg) | N/T | N/T | N/T | N/T | N/D | N/D |
| L-MTZ | Metronidazole(25µg) | 0 | - | 0 | 100 | | |

Key: S = Sensitive; I = Intermediate; R = Resistant; x = mean sensitive zone of inhibition; n = Total organisms tested; L = local brand; $S^c$ = standard commercial control; CIP – Ciprofloxacin; CXM – Cefuroxime; CLI – Clindamycin; FLU – Flocloxacillin; AMC – Amoxicillin; AMOC – Amoxyclav; MTZ – Metronidazole; N/D = Not done; N/A = Not tested

***Table 2:*** *Antimicrobial susceptibility patterns of MSSA against different standard controls and local brands antibiotics by the disc diffusion method and their mean susceptible zones of inhibition (n = 52).*

**Antibiotic susceptibility and resistance profile of Pseudomonas spp (ESBL and non ESBL producers)**

A total of fifty-nine confirmed Pseudomonas spp. isolates were tested for the presence of ESBL production using ceftazidime disc alone and ceftazidime plus clavulanic acid. Of these, 35% (n = 21) of the Pseudomonas spp. strains were detected to be ESBL positive. This is slightly higher than the prevalence of ESBL in pus (28.36%) reported by [Shaikh., *et al*. 2015] and lower than the 87.5% detected in [China by Chen., *et al*. 2015]. The low prevalence of ESBL detected in this study might be due to the low antibiotic usage in the rural areas where the study was conducted and the fact that majority of the patients who took part in this study were from the outpatient department.

ESBL antimicrobial resistance levels were very high (>50%) in all, except amoxiclav (48%) (Table 3). The local brand of the ciprofloxacin recorded the highest susceptible mean zone of inhibition of 29 ± 2.2mm, while the local brand of flucloxacillin obtained the lowest susceptible mean zone of inhibition of 21 ± 0mm (Table 3). The resistance conferred by ESBLs producing Pseudomonas spp. to clindamycin, cefuroxime, flucloxacillin, ciprofloxacin, and amoxicillin of the local antibiotics were 57%, 100%, 95%, 57%, and 76% respectively. Apart from cefuroxime there were no statistical significant differences ($p > 0.05$) between the susceptible mean zones of inhibition of the local brand of antibiotics and their respective standard controls (Table 3). Spanu., *et al*. (2002) reported that treatment of infection with ESBL-producing pathogens is more difficult to treat, due to the detection of plasmid-mediated resistance mechanism to antimicrobial classes other than β-lactams. These results are also in agreement with Newman., *et al*. (2015) where they detected 50% to 100% antimicrobial resistance among ESBL producers, but contrary to the findings of Ahmad., *et al*. (2016), where they observed 78.44% of ESBL resistance to amoxyclav.

| Code | Antibiotics | S (%) | X (mm) | I (%) | R (%) | T test value | P value |
|---|---|---|---|---|---|---|---|
| $S^c$-CIP | Ciprofloxacin (5µg) | 43 | 27 ± 1.4 | 0 | 57 | -0.305 | 0.762 |
| L-CIP | Ciprofloxacin (5µg) | 43 | 29 ± 2.2 | 0 | 57 | | |
| $S^c$-CXM | Cefuroxime (30µg) | 11 | 25 ± 2.1 | 0 | 89 | 3.902 | 0.000 |
| L-CXM | Cefuroxime (30µg) | 0 | - | 0 | 100 | | |
| $S^c$-CLI | Clindamycin (2µg) | 38 | 27 ± 3.5 | 0 | 62 | -0.104 | 0.918 |
| L-CLI | Clindamycin (2µg) | 43 | 28 ± 3.3 | 0 | 57 | | |
| $S^c$-FLU | Flucloxacillin (5µg) | 5 | 21 ± 0 | 0 | 95 | -0.274 | 0.785 |
| L-FLU | Flucloxacillin (5µg) | 5 | 21 ± 0 | 0 | 95 | | |
| $S^c$-AMC | Amoxicillin(20µg) | 14 | 22 ± 1.0 | 0 | 76 | -0.116 | 0.909 |
| L-AMC | Amoxicillin (20µg) | 14 | 21 ± 0.6 | 0 | 76 | | |
| $S^c$-AMOC | Amoxiclav(20/10µg) | 47 | 24 ± 1.3 | 5 | 48 | 0.178 | 0.860 |
| L-AMOC | Amoxiclav(20/10µg) | 47 | 23 ± 1.6 | 5 | 48 | | |
| $S^c$-MTZ | Metronidazole(25µg) | N/T | N/T | N/T | N/T | N/D | N/D |
| L-MTZ | Metronidazole(25µg) | 0 | 0 | 0 | 100 | | |
| S = Sensitive; I = Intermediate; R = Resistant; x = mean sensitive zone of inhibition; n = Total organisms tested; L = local brand; Sc = standard commercial control; CIP – Ciprofloxacin; CXM – Cefuroxime; CLI – Clindamycin; FLU – Flucloxacillin; AMC – Amoxicillin; AMOC – Amoxiclav; MTZ – Metronidazole; N/D = Not done; N/A = Not tested. | | | | | | | |

***Table 3:*** *Activities of the standard controls and local brands of antibiotics tested against Pseudomonas spp. (ESBL producers) and their mean susceptible zones of inhibition. (n = 21)*

Our result also indicated that some of the Pseudomonas spp. (non ESBL producers) has considerable levels of antibiotic resistance. There was resistance to a wide range of clinically relevant antimicrobial agents, including cefuroxime (89%), flucloxacillin (92%), and amoxicillin (71%) (Table 4). However, there were high susceptibility to ciprofloxacin (97%), clindamycin (82%) and amoxiclav (66%). [Raja., *et al*. 2007] reported of 88.7% susceptibility of ciprofloxacin to Pseudomonas spp. The local brand of metronidazole showed no potency (100% resistance) against all isolates tested. This is consistent with an earlier study by Modak., *et al*. (2015) but contrary to the work of [Akhi., *et al*. 2015] who demonstrated that anaerobic bacteria Clostridium perfringens and Bacteroides fragilis exhibited a susceptibility rate of 100% and 69.2% respectively to metronidazole. The discrepancies in the potency might be due to the testing of only aerobic bacteria in our study. Even though majority of the isolates were resistance to the local brand of cefuroxime, none were susceptible and few intermediate (Table 4). The present outcome is comparable to that of [Helegbe., *et al*. 2009] but contrary to a study conducted in both Ghana and Nigeria, where it was observed that, drugs from these two countries are substandard and do not comply with the US Pharmacopoeia standard [Egbo, 2013]. The mean zone of inhibition of local brand of cefuroxime was significantly lower ($p < 0.05$) than that of the standard commercial control disc and relates to the findings of [Nkang., *et al*. 2010].

| Code | Antibiotics | S (%) | X (mm) | I (%) | R (%) | T test value | P value |
|---|---|---|---|---|---|---|---|
| Sc-CIP | Ciprofloxacin (5µg) | 95 | 29 ± 3.4 | 5 | 0 | -1.663 | 0.101 |
| L-CIP | Ciprofloxacin (5µg) | 97 | 30 ± 3.2 | 3 | 0 | | |
| Sc-CXM | Cefuroxime (30µg) | 29 | 24 ± 1.1 | 0 | 71 | 5.138 | 0.000 |
| L-CXM | Cefuroxime (30µg) | 0 | - | 11 | 89 | | |
| Sc-CLI | Clindamycin (2µg) | 79 | 31 ± 3.4 | 5 | 16 | -0.647 | 0.520 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| L-CLI | Clindamycin (2µg) | 82 | 32 ± 3.4 | 8 | 10 | | |
| Sc-FLU | Flucloxacillin (5µg) | 11 | 24 ± 0.6 | 0 | 89 | -0.482 | 0.631 |
| L-FLU | Flucloxacillin (5µg) | 8 | 23 ± 1.0 | 0 | 92 | | |
| Sc-AMC | Amoxicillin (20µg) | 26 | 22 ± 1.1 | 0 | 74 | -0.056 | 0.955 |
| L-AMC | Amoxicillin (20µg) | 26 | 22 ± 1.2 | 3 | 71 | | |
| Sc-AMOC | Amoxiclav(20/10µg) | 66 | 24 ± 1.8 | 0 | 34 | -0.170 | 0.865 |
| L-AMOC | Amoxiclav(20/10µg) | 66 | 23 ± 1.6 | 0 | 34 | | |
| Sc-MTZ | Metronidazole(25µg) | N/T | N/T | N/T | N/T | N/D | N/D |
| L-MTZ | Metronidazole(25µg) | 0 | 0 | 0 | 100 | | |

S = Sensitive; I = Intermediate; R = Resistant; x = mean sensitive zone of inhibition; N = Total organisms tested; L = local brand; Sc = standard commercial control; CIP – Ciprofloxacin; CXM – Cefuroxime; CLI – Clindamycin; FLU – Flucloxacillin; N/D = Not done; N/T = Not tested

***Table 4:*** *Antimicrobial susceptibility patterns of Pseudomonas spp. (non ESBL producers) against different antibiotics by disc diffusion method (n = 38).*

## Conclusion

In conclusion, this study data show that most of the isolates were resistance to β-lactams antibiotics such as amoxicillin and flucloxacillin, whilst ciprofloxacin and clindamycin were very effective. It also indicated that there was a high prevalence of MRSA and ESBL while the overall susceptibility profile and mean zones of inhibition of most local brands of antibiotics tested were statistically comparable ($p > 0.05$) to the standard controls used.

### Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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