Original Research Article

ANTIBIOTIC SUSCEPTIBILITY PROFILE OF BACTERIA ISOLATED FROM FITNESS MACHINES IN SELECTED FITNESS CENTERS AT AKURE AND ELIZADE UNIVERSITY IN ONDO STATE NIGERIA.

ABSTRACT

Aim: This study seeks to determine the antibiotic susceptibility pattern of bacteria isolated from surfaces of fitness machines at gym center located at Elizade University and Akure town.

Methods: Samples were collected from different site of gym equipment including thread mill (handle, floor), bicep bench (handle), bike (handle, paddle), cruncher (handle, elbow) using sterile swab stick moistened with sterile buffered physiological solution. The swab sticks were immediately transferred to the laboratory for analysis. Standard microbiological techniques were used to identify the ~~B~~bacterial isolates. The antibiotic susceptibility profile of the isolates was determined by using standard antibiotics discs.

Result: Out of the 31 isolates identified, *Staphylococcus aureus* 12(38.7%) was ~~is~~ the predominant bacteria followed by *Bacillus* spp. 11(35.5%), *Klebsiella* spp. 4(12.9%), *E.coli* and *Staphylococcus saprophyticus* 2(6.45%) and *Enterococcus* spp. 1(3.23%). The susceptibility profile showed that all isolates were ~~are~~ resistant to Amoxicillin (AM) and Augmentin (AU), *Staphylococcus* spp. isolated from different surfaces shows different susceptibility pattern to the used antibiotics, while *Bacillus* spp. *Klebsiella* spp. and *E. coli* also confer resistance to more than one commonly used antibiotic.

Conclusion: The results showed occurrence of potential pathogenic bacteria in which their presence on the equipment surfaces could easily be transmitted between users and to the environment generally. The spread of these potential pathogenic microorganisms in the fitness centre can be prevented through frequent hand washing and use of hand sanitizer as well as daily cleaning of equipment surfaces before and after activities with disinfectants.

Keywords: fitness center, gym center, antimicrobial resistance, fomites, fitness equipment

INTRODUCTION

Public fitness center, also commonly referred to as "gym center" provides a wide range of exercise equipment for use by people.ref Exercise equipment provides a whole lot of health benefits including keeping fit, losing excessive weight, reducing depression, stress etc (Zina *et al*., 2018). It is progressively becoming a tradition in different parts of Nigeria to have people spending time at the gym center particularly during weekends and sometime during the week days. ~~An a~~Average Nigerians have began to see the act of visiting fitness centers as a good lifestyle which is in no doubt a welcome development. However, little is known about the potential of the transmission of infectious microbial agents among users within the fitness centers. Frequently touched surfaces of public places have been shown to abhor significantly

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high population of microorganisms that are known to be normal flora found in humans (Mukherjee, 2014). Previous studies have reported the contamination of various indoor environments due to microorganisms released by humans (National Academic of Sciences, Engineering and Medicine, 2017). Studies have also shown that bacterial species found on public surfaces are those that are associated with the normal flora of the skin and body because of constant contact with the hands and faces (Chengula *et al*., 2014; Wood *et al*., 2015).

Marianne *et al*., 2017 in their study revealed the occurrence of resistance strains of bacteria on surfaces of fomites. Previous studies have revealed the major concerns associated with use of antibiotic which is the emergence of resistant strains of microorganisms, majority of which have developed resistance to almost all of the commonly used antibiotics, and these poses as public health concerns (Davies *et al*, 2010).

A lot of studies ref have been carried out to determine the possible means that infection can be spread in the environment. Study on money, swimming pool, markets, ATM machines, associations between human use and bacterial community composition on kitchen surfaces, with bacterial taxa commonly found on human skin predominating on kitchen surfaces, consistent with frequent skin to surface contact (Meadow *et al*., 2014).

While volumes of studies have revealed the burden of AMR within hospitals and other built environments (Orji *et al*., 2005; Russotto *et al*., 2015; Monegro *et al*., 2017), much is yet to be unveiled about the occurrence and or the prevalence of AMR bacterial strains on surfaces of fitness equipments within public fitness centers. This study is aimed at determining the occurrence of antibiotic resistant bacteria on surfaces of fitness machines found at gym centers.

2. MATERIALS AND METHOD

2.1 Study area and study design

Total of 2 gym centers situated within Elizade University campus and Akure town respectively were used in this study. Both centers are equipped with modern fitness machines which include; Cruncher, exercise bike (out of use at Akure center), Treadmill, bicep bench, dumbbell, barbells, AB lounge and host of other minor exercise equipments.

Prior to sample collection, few observations were made around and within the premises of the fitness centers. The Elizade University environment ~~U~~ unlike the Akure town is devoid of straying animals like dogs, goat, chickens and ~~S~~ sheep. A lot of animal's droppings were sighted around the compound of the gym center located in Akure town. The gym situated within the Elizade University campus records high level of usage compare to the one situated within Akure metropolis. Record as shown at the respective gym centers indicates that certain fitness machines were frequently used by male compared to female while some were also frequently used by female than the male; the stationary Bike, the Cruncher and the Treadmill were frequently used by females while the bicep bench and AB lounge is frequently used by the male.

Samples were collected at peak period during use. Machines to be sampled were selected based on frequency of use.

2.2. Sample analysis

The equipment and sites where the samples were collected includes the following, thread mill (handle, floor), bicep bench (handle), exercise bicycle (handle and pedal), and cruncher (handle and elbow). Each target site was swabbed with 4 different swab sticks for each type of a selected culture media. The sites were swabbed with moistened sterile cotton-tipped swab and carefully immersed into the plastic test tube that contains 1 mL of sterile tryptic soy broth which was immediately taken to the laboratory for microbiological analysis.

2.3. Sample processing

Swabbed samples were inoculated unto their respective media including Mannitol Salt Agar (Oxoid, England), Eosin Methylene Blue Agar (BBL™, USA) and Salmonella Shigella Agar (Oxoid, England); the media were prepared following the manufacturers' instruction. Inoculated plates were incubated at 37ºC for 24 h to 48 h, after which the plates were observed for growth and colony morphology. The presumptive identification of the isolates was made based on the colony morphology and Gram's reaction. The identities of the pure bacterial isolates were confirmed based on the enzyme activities and biochemical characteristics. All tests that were carried out were done following standard microbiological protocol as described by Cheesebrough, 2005.

2.4. Antibiotics sensitivity test

Antimicrobial susceptibility testing was performed for each of the bacterial isolates using Mueller Hinton Agar (MHA) (Oxoid, England) by the Kirby–Bauer disc diffusion method following standard procedures. ref A suspension of each of the bacterial isolate was prepared whilst adjusted to 0.5 McFarland. A sterile cotton swab was used to collect bacterial suspension remove the excess suspension by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of MHA. The inoculated plates were left at room temperature to dry for 3 to 5 min, and a set of antibiotic discs were placed on the inoculated plates aseptically, using sterile forceps and were allowed to stand for 30 min after which the plates were incubated for 16 to 18 h at 35°C. After incubation, the zones of inhibition were measured using a ruler. The diameters of the zones of inhibition for each isolates and antibiotic used were further interpreted according to the standards as provided by Clinical and Laboratory Standards Institute (CLSI).ref whch year? The antimicrobial discs used for susceptibility testing includes the following; Ciprofloxaxin (CPX, 10 µg), Septrin (SXT, 30 µg), Gentamycin (CN, 10 µg), Streptomycin (S, 30 µg), Amoxycillin (AM, 30 µg), Erythromycin (E, 10 µg), Augmentin (AU, 30 µg), Tarivid (OFX, 10 µg), Chloranphenicol (CH, 30 µg) ref

2.5. Data analysis

Data obtained from the microbiological analysis were analyzed using SPSS 21 version.

3. RESULT

In this study, a total of 31 isolates picked at random were identified where 15 and 16 were obtained from the Elizade University and Akure center respectively (**Table 1)**. A total of 29 were picked for the determination of the Antibiotic sensitivity pattern **(Figure 1 and 2)**. Out of the 31 isolates identified, *Staphylococcus aureus* 12(38.7%) showed to be the predominant bacteria followed by *Bacillus* spp. 11(35.5%), *Klebsiella* spp. 4(12.9%), while *E.coli* and *Staphylococcus saprophyticus* 2(6.45%) and *Enterococcus* spp. 1(3.23%).

Table 1. Identified bacteria isolated from the two fitness centers, 2018

Fitness machine	**Bacteria identified at the 2 fitness centers**	
	Elizade University	**Akure town**
Bicycle pedal (BP)	*Staphylococcus aureus, Staphylococcus saprophyticus Bacillus* spp.	MOU
Bicycle handle (BH)	*Staphylococcus aureus,*	MOU
Treadmill handle (TMH)	*Staphylococcus aureus, Bacillus* spp.	*Staphylococcus aureus, Klebsiella* spp *Bacillus* spp.
Treadmill floor (TMF)	*Staphylococcus aureus Staphylococcus saprophyticus Bacillus* spp.	*Bacillus* spp. *E.coli*
Cruncher Handle (CH)	*Staphylococcus aureus Bacillus* spp.	*Staphylococcus aureus Bacillus* spp. *Enterococcucs* spp.
Cruncher elbow (CE)	*Staphylococcus aureus Bacillus* spp.	*Staphylococcus aureus Klebsiella* spp.
AB lounge pedal (ABP)	*Staphylococcus aureus Bacillus* spp.	*Klebsiella* spp. *E.coli*
Door Handle (Main entrance)	*Staphylococcus aureus Bacillus* spp.	*Staphylococcus aureus Klebsiella* spp *Bacillus* spp.

MOU – Machine out of use.

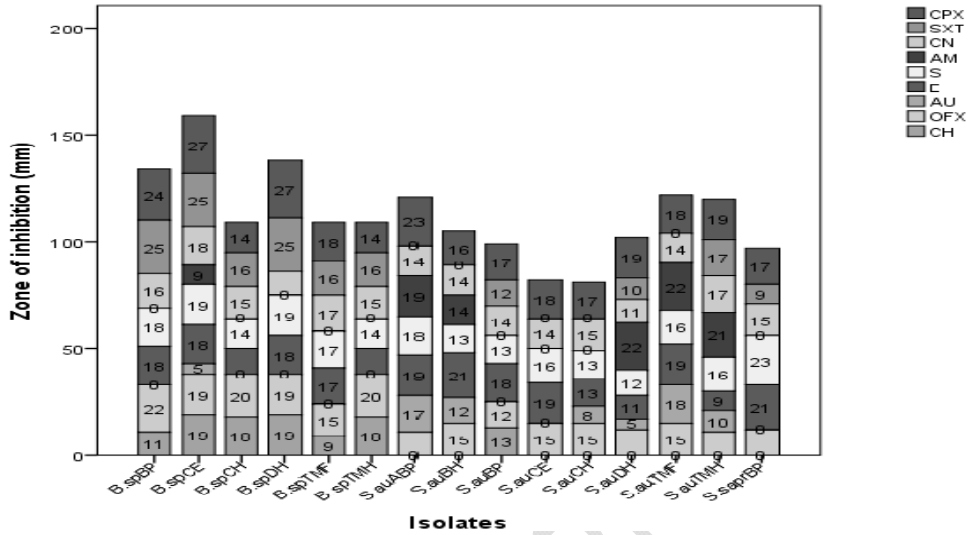

Figure1. Susceptibility pattern of bacteria isolated from fitness machines at Elizade University's gym Centre. **S.au-** *Staphylococcus aureus*, **S.sapr** - *S. saprophyticus*, **B.sp-** *Bacillus* sp. **BP-** Bike pedal, **DH-** Door Handle, **TMH-** Thread-mill Handle, **TMF-** Thread-mill Floor, **CH-** Cruncher handle, **CE-** Cruncher elbow, **ABP-** AB lounge Pedal, **DH-** Door handle. **CH-** Chloramphenicol, **OFX-** Ofloxacin, **AU-** Augmentin, **E-** Erythromycin, **S-** Streptomycin, **AM-** Amoxacillin, **CN-** Gentamycin, **SXT-** Septrin, **CPX-** Ciprofloxacin.

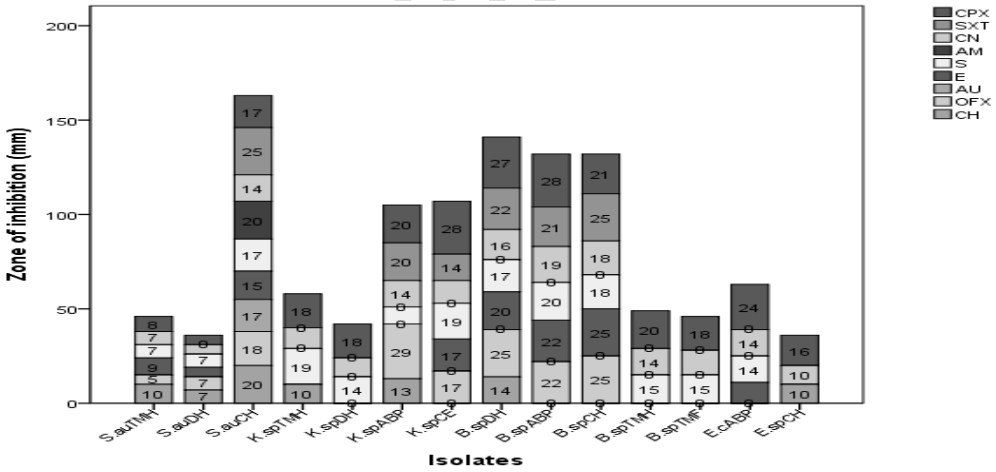

Figure 2: Susceptibility pattern of bacteria isolated from fitness machines in gym Centre located at Akure. S.au- *Staphylococcus aureus,* K.sp- *Klebsiella* sp, B.sp-*Bacillus* sp,. E.c- *Escherichia coli*, E.sp- *Enterobacter* sp. **TMH-** Treadmill Handle, **DH-** Door Handle, **ABP-** Abdominal lounge Pedal, **CE-** Cruncher Elbow. **CH-** Chloramphenicol, **OFX-** Ofloxacin, **AU-** Augmentin, **E-** Erythromycin, **S-** Streptomycin, **AM-** Amoxacillin, **CN-** Gentamycin, **SXT-** Septrin, **CPX-** Ciprofloxacin.

The distribution of bacteria as identified in the two centers differs; *Klebsiella* spp., *Enterococcucs* spp. and *E.coli*, were isolated from the Akure but was absent in the samples obtained from the Elizade University center. On the other hand, *S. Saprophyticus* was isolated from the Elizade University, but was absent from the samples obtained from the Akure center.

Result of the antibiotic susceptibility test as obtained showed that bacteria of the same genus and specie isolated from surfaces of fitness machines at the same center have different susceptibility pattern to identical antibiotics used **Figure 1 and 2.**

Nine commonly used antibiotics were used in this study to evaluate the susceptibility pattern of the bacterial isolate. The result as obtained indicates that several of the isolates showed zone of inhibition against more than one antibiotic **Figure 1 and 2.** However, according to the AST interpretative chart (CLSI, 2014), all the isolates showed resistance to more than one antibiotics **Table 2 and 3**.

Table 2: Interpretation of the antimicrobial susceptibility test result at Elizade University.

S/N	Isolates	CPX	SXT	CN	S	AM	E	AU	OFX	CH
1	*S. aureus* **BP***	I	I	R	R	S	I	S	R	R
2	*S. saprophyticus* **BP***	I	R	I	S	R	I	R	R	R
3	*Bacillus* sp. **BP***	S	S	S	I	R	I	R	S	R
4	*S. aureus* **TMH***	I	S	S	I	S	R	S	R	R
5	*S. aureus* **DH***	I	R	R	R	S	R	R	R	R
6	*S. aureus* **CH***	I	R	I	R	R	I	R	I	R
7	*S. aureus* **CE***	I	R	I	R	R	I	R	I	R
8	*S. aureus* **BH***	R	R	R	I	R	R	R	R	R
9	*Bacillus* sp. **TMH***	I	S	I	I	R	I	R	S	S
10	*Bacillus* sp. **DH***	I	S	R	I	R	R	R	I	I
11	*S. aureus* **ABP***	S	R	I	I	S	I	S	R	R
12	*Bacillus* sp. **CH***	R	S	I	R	R	R	R	S	S
13	*Bacillus* sp. **TMF***	S	S	S	I	R	I	R	I	S
14	*Bacillus* sp. **CE***	S	S	S	I	R	I	R	I	S
15	*S. aureus* **TMF***	I	R	I	I	S	I	S	I	R

Resistance (R), Intermidiate (I), Susceptible (S), *- Site of sample collection see Figure 1

Table 3: Interpretation of the antimicrobial profile from Akure town

S/N	Isolates	CPX	SXT	CN	S	AM	E	AU	OFX	CH
1	*S. aureus* **TMH***	R	R	R	R	R	R	R	R	R
2	*S. aureus* **DH***	R	R	R	R	R	R	R	R	R
3	*S. aureus* **CH***	**S**	**S**	**I**	**S**	**S**	**S**	**S**	**S**	**S**
4	*Klebsiella* sp. **TMH***	I	R	R	I	R	R	R	R	R
5	*Bacillus* sp. **DH***	S	S	S	I	R	I	R	S	I
6	*Klebsiella* sp. **DH***	I	R	R	R	R	R	R	R	R
7	*Klebsiella* sp. **ABP***	I	S	R	R	R	R	R	S	R
8	*Bacillus* sp. **ABP***	S	S	S	I	R	I	R	S	R
9	*Escherichia coli* **ABP***	S	R	I	R	R	R	R	R	R
10	*Bacillus* sp. **CH***	I	S	S	I	R	S	R	S	R
11	*Enterobacter* sp. **CH***	I	R	R	R	R	R	R	R	R
12	*Bacillus* sp. **TMH***	I	R	R	R	R	R	R	R	R
13	*Bacillus* sp. **TMF***	I	R	R	R	R	R	R	R	R
14	*Klebsiella* sp **CE***	S	I	R	I	R	I	R	I	R

Resistance (R), Intermediate (I), Susceptible (S) *- Site of sample collection see figure 2

4. DISCUSSION

The increasing prevalence and spread of antimicrobial resistant (AMR) strains of bacteria is evidently threatening the capacity of treating infectious diseases. In effect, this poses a significant burden on public health. Volumes of studies ~~y~~ have revealed the prevalence and or the occurrence of MDR/AMR microorganisms in clinical environment such as the hospitals.ref Previous study has also shown the occurrence of these organisms in other indoor built environment like the care homes, nursery, kitchen, offices, laboratories etc.ref Less is known about occurrence of and transmission of MDR/AMR in the fitness centers. Attention is now drawn to the non-clinical environment such as the gym centers which has the potential to equally play a significant role in the spread of infectious antibiotic resistant microorganisms. It has been established in previous studies that surfaces of fomites spread of infectious disease, and studies have also shown that the spread of the these infectious diseases are associated with human that has been exposed to indoor pathogens (Dick *et al.,* 1987; Wong *et al.,* 2010).

The isolates in this study predominantly belongs to two (2) phylla; the firmicutes and the proteobacteria which correlates with the findings of Mukherjee *et al.*, (2014). *Staphylococcus aureus* constitute the major isolates in this study, this may be due to frequent contact with

machines by users as it is well established that the bacteria is commonly associated with human flora. *Bacillus* sp. is another bacterium that was isolated from both center and which is commonly found in the soil. Interestingly, *Staphylococcus saprophyticus* was isolated from the sample obtained from the Bicycle pedal in the gym center situated within the Akure town but not detected in samples obtained from the Elizade University gym center. *S. saprophyticus* has been isolated from animal stools and is known to be human as part of the normal flora of the female genital tract and perineum (Widerström *et al.,* 2012). It has also been reported to cause uncomplicated urinary tract infection in sexually active women (Eriksson *et al.,* 2013). These coagulase negative bacteria in this study showed resistance to Septrin (Trimethoprim/ Sulfamethoxazole), Ampicillin, Augmentin, ~~o~~ Ofloxacin and Chloramphenicol. Although, complicated cases of urinary tract infection caused by *S. saprophyticus* has usually been treated with trimethoprim-sulfamethoxazole. However, as evidenced in this study, previous work has reported resistance of *S. saprophyticus* to trimethoprim-sulfamethoxazole (De Sousa *et al.,* 2017). Its presence on the BP can be attributed to contact with contaminated soil via foot wears of users. *Bacillus* species isolated from Bike pedal, thread mill handle and door handle have a similar susceptibility pattern, except for the one isolated from door handle which shows resistance to Gentamycin.

Other isolates including *E.coli, Enterobacter* spp. and *Klebsiella* spp. isolated from samples obtained in the gym center located within the Akure metropolis also conferred resistance to multiple common antibiotics used in this study. These organism as earlier mentioned in this paragraph are members of the enterobacteriaceae which source is suggestive of intestinal origin. In effect, indicating evidence of fecal contamination. As part of the observation that was made at both centers, ruminant animal and poultry droppings (faeces) were sighted at the premise of gym center located the Akure town, but none was spotted at the center located at Elizade University campus. The campus is devoid of free range poultry and ruminant animals as the University's policy prohibit such activities. A previous study has shown that environmental conditions and hygiene of fitness centers which is very crucial to exercisers' health has a major role to play in the occurrence and spread of infectious diseases (Onchang and Panyakapo, 2014).

The genus/specie composition of the bacteria isolated from the University's gym center differs from that obtained at the center in Akure speaks volume about what factors determines the occurrence of population of microorganisms ~~**Table 1**~~. The variation as evidenced in this study is in tandem with previous studies which shows that population and or the specie composition of microorganisms found in built indoor environment is determined by the mixture of microbes present in the immediate outdoor environment and those carried by people and their pets/animals entering or living within the premise (Mukherjee *et al*., 2014).

Transmission of AMR within non-clinical indoor environment like gym centers, playgrounds, schools, daycare centers, prison jails and athletic facilities have been reported (David *et al.,* 2008; Montgomery *et al.,* 2010; Ryan *et al.,* 2011). Much is required to be done to intensify

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efforts for the surveillance of AMR within non-clinical indoor environment particularly the fitness centers.

5. CONCLUSION

Conclusively, fitness centers with all the facilities in place are in no doubt remains a vital place to visit to ensure body fitness and reduce risk of health concerns and diseases. However, gym center owners are advised to ensure health and safety of their clients by ensuring to establish and maintain a hygiene environment of the fitness equipment. Users should be aware of the danger inherent in not paying attention to the potentials of the transmission of infectious diseases within gym centers. It has been established in this study that fitness center is an unnoticed and potential source of transmission of community acquirable antibiotic resistant strains of bacteria.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare

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