

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 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%) 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 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 result 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. 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 part of Nigeria to have people spending time at the gym center particularly during weekends and sometime during the week days. An 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 harbor significantly high population of microorganisms that are known to be normal flora found in human (Mukherjee, 2014). Previous

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

44 Marianne *et al.*, 2017 in their study revealed the occurrence of resistance strains of bacteria on
45 surfaces of fomites. Previous studies have revealed the major concerns
46 associated with use of antibiotic which is the emergence of resistant strains of
47 microorganisms, majority of which have developed resistance to almost all of the
48 commonly used antibiotics, and these poses as public health concerns (Davies *et al.*,
49 2010).

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

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

60 **2. MATERIALS AND METHOD**

61 **2.1 Study area and study design**

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

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

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

77 **2.2. Sample analysis**

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

84 **2.3. Sample processing**

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

94 **2.4. Antibiotics sensitivity test**

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

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112 **2.5. Data analysis**

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

114 **3. RESULT**

115 In this study a total of 31 isolates picked at random were identified where 15 and 16 were obtained from
 116 the Elizade University and Akure center respectively (**Table 1**). A total of 29 were picked for the
 117 determination of the Antibiotic sensitivity pattern (**Figure 1 and 2**). Out of the 31 isolates identified,
 118 *Staphylococcus aureus* 12(38.7%) showed to be the predominant bacteria followed by *Bacillus* spp.
 119 11(35.5%), *Klebsiella* spp. 4(12.9%), while *E.coli* and *Staphylococcus saprophyticus* 2(6.45%) and
 120 *Enterococcus* spp. 1(3.23%).

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

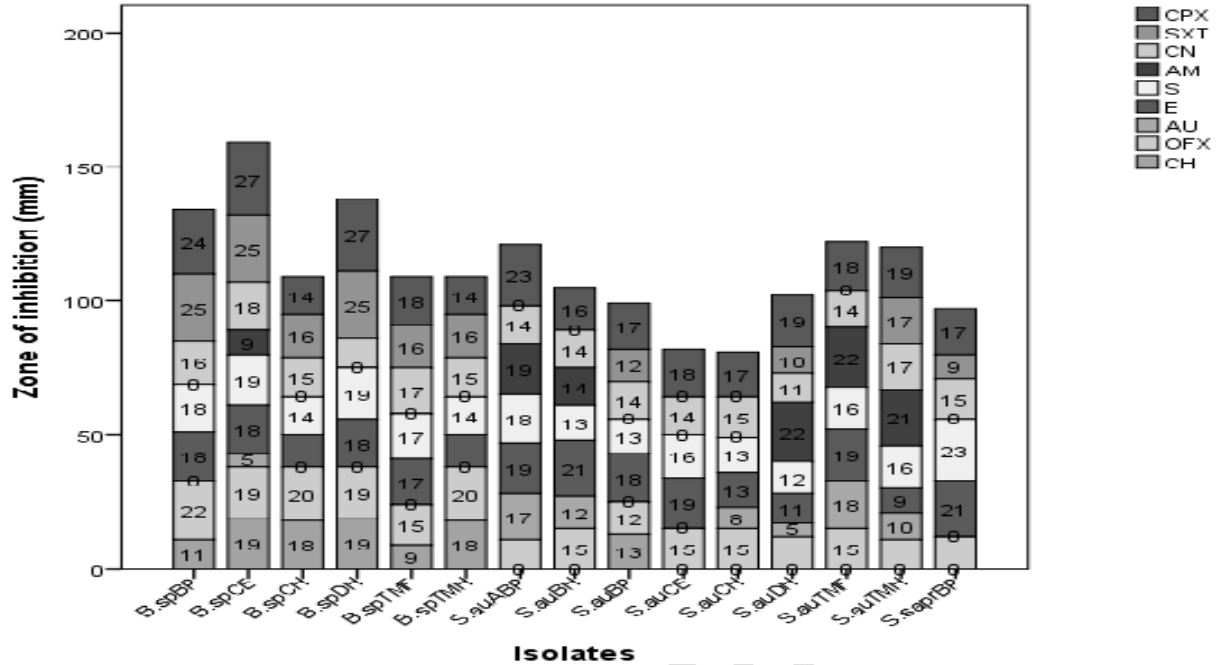
Fitness machine	Bacteria identified at the 2 fitness centers	
	Elizade University	Akure town
Bicycle pedal (BP)	<i>Staphylococcus aureus</i> , <i>Staphylococcus saprophyticus</i> <i>Bacillus</i> spp.	MOU
Bicycle handle (BH)	<i>Staphylococcus aureus</i> ,	MOU
Treadmill handle (TMH)	<i>Staphylococcus aureus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

122 MOU – Machine out of use.

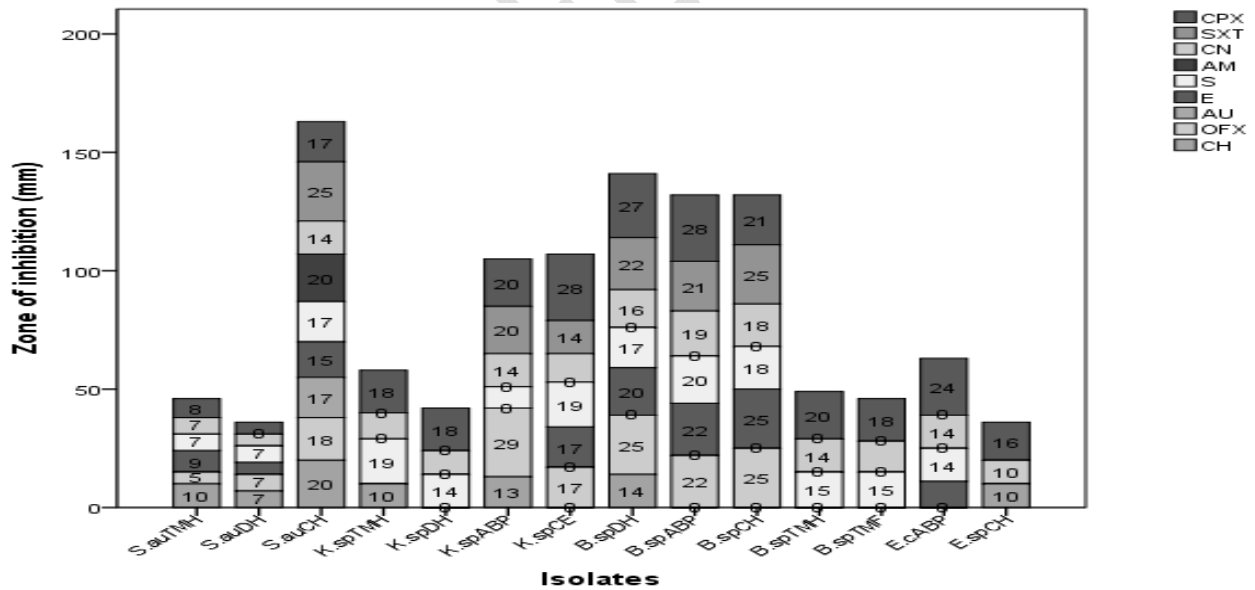
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 127 **Figure1.** Susceptibility pattern of bacteria isolated from fitness machines at Elizade University's gym
 128 Centre. **S.au-** *Staphylococcus aureus*, **S.sapr** - *S. saprophyticus*, **B.sp-** *Bacillus* sp. **BP-** Bike pedal, **DH-**
 129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



133
 134 **Figure 2:** Susceptibility pattern of bacteria isolated from fitness machines in gym Centre located at
 135 Akure. **S.au-** *Staphylococcus aureus*, **K.sp-** *Klebsiella* sp, **B.sp-** *Bacillus* sp,. **E.c-** *Escherichia coli*, **E.sp-**
 136 *Enterobacter* sp. **TMH-** Treadmill Handle, **DH-** Door Handle, **ABP-** Abdominal lounge Pedal, **CE-**
 137 Cruncher Elbow. **CH-** Chloramphenicol, **OFX-** Ofloxacin, **AU-** Augmentin, **E-** Erythromycin, **S-**
 138 **Streptomycin, AM-** Amoxicillin, **CN-** Gentamycin, **SXT-** Seprin, **CPX-** Ciprofloxacin.

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

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

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

151 **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	<i>S. aureus</i> BP*	I	I	R	R	S	I	S	R	R
2	<i>S. saprophyticus</i> BP*	I	R	I	S	R	I	R	R	R
3	<i>Bacillus</i> sp. BP*	S	S	S	I	R	I	R	S	R
4	<i>S. aureus</i> TMH*	I	S	S	I	S	R	S	R	R
5	<i>S. aureus</i> DH*	I	R	R	R	S	R	R	R	R
6	<i>S. aureus</i> CH*	I	R	I	R	R	I	R	I	R
7	<i>S. aureus</i> CE*	I	R	I	R	R	I	R	I	R
8	<i>S. aureus</i> BH*	R	R	R	I	R	R	R	R	R
9	<i>Bacillus</i> sp. TMH*	I	S	I	I	R	I	R	S	S
10	<i>Bacillus</i> sp. DH*	I	S	R	I	R	R	R	I	I
11	<i>S. aureus</i> ABP*	S	R	I	I	S	I	S	R	R
12	<i>Bacillus</i> sp. CH*	R	S	I	R	R	R	R	S	S
13	<i>Bacillus</i> sp. TMF*	S	S	S	I	R	I	R	I	S
14	<i>Bacillus</i> sp. CE*	S	S	S	I	R	I	R	I	S
15	<i>S. aureus</i> TMF*	I	R	I	I	S	I	S	I	R

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

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158 **Table 3:** Interpretation of the antimicrobial profile from Akure town

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

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

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161 4. DISCUSSION

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

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

177 machines by users as it is well established that the bacteria is commonly associated with human
178 flora. *Bacillus* sp. is another bacterium that was isolated from both center and which is
179 commonly found in the soil. Interestingly, *Staphylococcus saprophyticus* was isolated from the
180 sample obtained from the Bicycle pedal in the gym center situated within the Akure town but not
181 detected in samples obtained from the Elizade University gym center. *S. saprophyticus* has been
182 isolated from animal stools and is known to be human as part of the normal flora of the female
183 genital tract and perineum (Widerström *et al.*, 2012). It has also been reported to cause
184 uncomplicated urinary tract infection in sexually active women (Eriksson *et al.*, 2013). These
185 coagulase negative bacteria in this study showed resistance to Septrin (Trimethoprim/
186 Sulfamethoxazole), Ampicillin, Augmentin, ofloxacin and Chloramphenicol. Although,
187 complicated cases of urinary tract infection caused by *S. saprophyticus* has usually been treated
188 with trimethoprim-sulfamethoxazole. However, as evidenced in this study, previous work has
189 reported resistance of *S. saprophyticus* to trimethoprim-sulfamethoxazole (De Sousa *et al.*,
190 2017). Its presence on the BP can be attributed to contact with contaminated soil via foot wears
191 of users. *Bacillus* species isolated from Bike pedal, thread mill handle and door handle have a
192 similar susceptibility pattern, except for the one isolated from door handle which shows
193 resistance to Gentamycin.

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

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

212 Transmission of AMR within non-clinical indoor environment like gym centers, playgrounds,
213 schools, daycare centers, prison jails and athletic facilities have been reported (David *et al.*,
214 2008; Montgomery *et al.*, 2010; Ryan *et al.*, 2011). Much is required to be done to intensify

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

217 **5. CONCLUSION**

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

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228 **CONFLICT OF INTEREST**

229 The authors have no conflict of interest to declare

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