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Antibiotics Susceptibility of *Staphylococcus aureus* isolates from the Lecture Theatres of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

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ABSTRACT

In recent time *Staphylococcus aureus* has developed resistance to commonly used antibiotics making its infections difficult to treat. The evaluation of microorganisms present in indoor environments especially lecture rooms is very critical considering the huge number of students involved. This study was designed to isolate *Staphylococcus aureus* from the lecture theatres of faculty of Pharmaceutical Sciences, Ahmadu Bello University and to determine their antibiotics susceptibility. Samples were collected from the floors, tables, door and indoor air of the theatres and were identified using standard microbiological techniques. Kirby Bauer disc diffusion method was used for antibiotics susceptibility testing. Fifty seven (57) samples were collected of which 26 (45.6 %) were *S. aureus*, 17 (29.8 %) were coagulase negative Staphylococci and 14 (24.6 %) were *Streptococci spp*. All the *S. aureus* isolates were resistant to amoxicillin (100 %), and were found to have susceptibility of 73.1 %, 69.2 %, 26.9 %, 23.1 % and 19.2% to chloramphenicol, gentamicin, tetracycline, trimethoprim and erythromycin respectively. Multidrug resistance observed was 73.1 % while pandrug resistance was 3.5 %. In conclusion there was a high prevalence of multidrug resistant *S. aureus* in the lecture theatres of Faculty of Pharmaceutical Sciences, Ahmadu Bello University.

Keywords: multidrug resistance, lecture theatre, *Staphylococcus aureus*

INTRODUCTION

Bacteria are microorganisms found everywhere in the universe, they are either pathogenic or non-pathogenic with the pathogenic ones being very harmful and can even lead to the death of the affected individuals (Ingraham, 2010). *Staphylococcus aureus* is a Gram positive cocci bacterium, a member of firmicutes (containing cell wall), belonging to the Micrococcaceae family. It is frequently found as commensal on skin, nose and mucous membranes of healthy humans, it is also a normal inhabitant of the lower reproductive tract of women. Human population are estimated as 20-30% long-term carriers of *S. aureus.* It is a common cause of illnesses like skin infections such as pimples, impetigo, boils, cellulitis, meningitis, folliculitis, carbuncles, osteomyelitis, staphylococcal scalded skin syndrome and life threatening diseases such as toxic shock syndrome, bacteremia and sepsis. It is one of the five most common causes of hospital acquired infections and is often the cause of wound infections following surgery (Rasigade *et al.,* 2014). Up to 50,000 deaths each year in the USA are linked with *S. aureus* infections (Athena *et al.,* 2019). The environment plays an important role in *S. aureus* transmissions and infections not only in healthcare settings but also in some high risk community settings including lecture theatres, households, prisons and public places. It does not normally cause infection on healthy skin; however *S. aureus* may breach innate host defense and gain access to deeper tissues causing a variety of superficial and invasive infections. (Plano *et al.,* 2013). One common mode of transmission of infections and microorganisms is through contact with surfaces such as table top, door knobs or handles, banisters and indoor air. A lecture hall (theatre) is a large room used for instruction typically at a college or university. Lecture halls almost always have a pitched floor, so that those in the rear sat higher than those at the front to allow them to see the lecturer. *S. aureus* infection may spread through contact with pus from an infected wound, skin to skin contact with an infected person by producing hyaluronidase that destroy that tissue (Harrison *et al.,* 2013). Transmission can also be through contact with objects such as towel, clothing or equipment used by an infected person. *S. aureus* can survive in air, objects and dry surfaces for days and months. As such they can contaminate the environment and are transmitted over a long period of time (Kramer, 2006; Brent *et al.,* 2019).

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S. aureus infections are common both as community acquired and hospital acquired and the treatment remains a challenge due to the emergence of multidrug resistant (MDR) strains such as methicillin-resistant *Staphylococcus aureus* (MRSA). An increased in the prevalence of methicillin resistance over the past four decades has led to high morbidity, mortality and increase in hospital expenditures associated with S. aureus infections. (Athena *et al.,* 2019). The increasing widespread use of antibiotics have led to the rapid appearance of antibiotic resistant strains today (Holden *et al.*, 2013). MDR *S. aureus* infections appear to be increasing and are displaying resistance to a wide range of antibiotics. European Center for Disease Control (ECDC) and Center for Disease Control (CDC), Atlanta have defined multidrug-resistant, extensively drug resistant (XDR) and pandrug resistant (PDR) (CDC, 2013). MDR was defined as acquired non susceptibility to at least one agent in three or more antimicrobial categories; XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories; PDR was defined as non-susceptibility to all agents in all antimicrobial categories (Magiorakos *et al.,* 2012). The aim of this study was to isolate S. aureus from the tables, floors, doors, and indoor air of the lecture theatres of the faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and to determine their susceptibility to commonly prescribed antibiotics.

MATERIALS AND METHOD

Sample collection

Samples were collected from the three lecture theatres of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria [Pa-Ishaku, Gabriel Osuidi Lecture theatres 1 and 2]. Swab samples were collected from floors, tables, door handles and door glasses of the three lecture theatres. The swab sticks were inoculated into peptone water and incubated at 37^{o}C for 24 hours. This was sub cultured into mannitol salt agar and incubated at 37^{o}C for 24 hours. To sample for the indoor air, mannitol salt agar plate was exposed at the center of each lecture theatre for 30 minutes, the plates were incubated at 37^{o}C for 30 minutes. Distinct colonies were transferred to nutrient agar slants and incubated at 37^{o}C for 24 hours. The slants were kept in the fridge for further tests.

Gram Staining and biochemical tests

Gram staining was performed on pure colonies of the samples as described by Cheesbrough (2002) and was examined under microscope using oil immersion under x100 magnification. Catalase and coagulase tests were performed to identify the *S. aureus* isolates, the procedures were carried out according to Cheesbrough (2002).

Antibiotics Susceptibility Testing

This was carried out using Kirby Bauer discs diffusion method, the interpretation of the inhibition zone diameter (millimeter) was done according to CLSI, (2013). The following commonly prescribed antibiotics were tested: Gentamicin 30 µg, tetracycline 30 µg, erythromycin 15 µg, trimethoprim 5 µg, chloramphenicol 30 µg and amoxicillin 30 µg. Multiple antibiotics resistance index (MARI) was calculated by dividing the number of antibiotics the isolate is resistant to by the total number of antibiotics tested (Krumpernum, 1993).

MDR, XDR and PDR strains were detected according to ECDC and CDC criteria (Magiorakos *et al.,* (2012).

RESULTS

A total of 57 samples were collected from the floors, doors, tables and indoor airs of the lecture theatres as shown in Table 1.

Table 1: Samples collected from the three lecture theatres of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria

Lecture theatres	Floor samples	Table samples	Indoor air	Door samples
Pa-Ishaku	6	10	1	2
GOLT-1	6	10	1	2
GOLT-2	6	10	1	2
Total	18	30	3	6

Keys: GOLT-1: Gabriel Osuidi Lecture theatre 1; GOLT-2: Gabriel Osuidi Lecture theatre 2

Table 2: Distribution of isolates in the lecture theatres

Lecture Theatre	S. aureus	CoNS	Streptococci	Total
Pa-Ishaku	8	5	6	19
GOLT -1	9	5	5	19
GOLT -2	9	4	6	19
Total	26	14	17	57

Keys: GOLT-1: Gabriel Osuidi Lecture theatre 1; GOLT-2: Gabriel Osuidi Lecture theatre 2

The result of the antibiotics susceptibility showed that chloramphenicol and gentamicin were the most active antibiotics while amoxicillin was the most inactive (Figure 1); 73.1 % were multidrug-resistant (Figure 2)

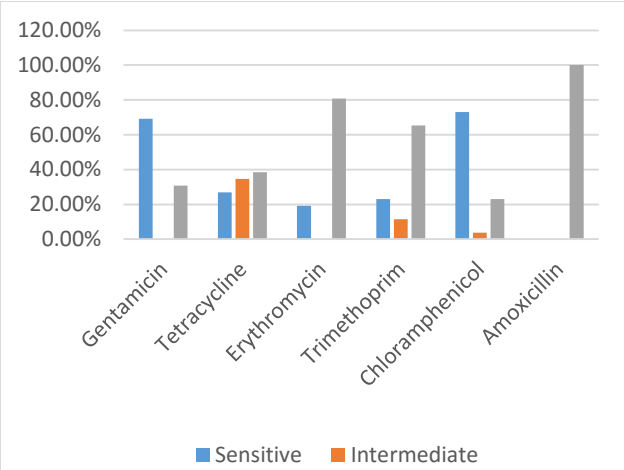

Figure 1: Percentage susceptibility profile of *S. aureus* isolates from the three lecture theatres

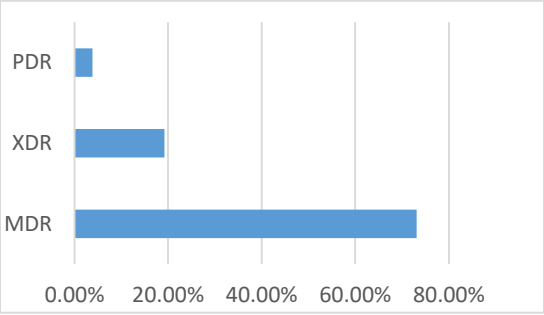

Figure 2: Incidence of MDR, XDR and PDR among *S. aureus* isolates from the lecture theatres

All the *S. aureus* isolates except one had MARI greater than 0.2

DISCUSSION

The evaluation of microorganisms present in indoor environments especially lecture rooms is very critical considering the huge number of students involved. The floors and the tables were the most contaminated. Shoe soles have been shown to transfer infectious microorganisms to floor and ground surfaces and these microorganisms can be transferred to man. The floor is the most exposed to contamination, shoe soles (bottom) has been implicated as potential vector (Rashid *et al.*, 2018); many of the most common microbiologic pathogens including MRSA, Enterococcus, *Cl. difficile*, and Gram-negative bacteria were identified on shoe soles (Chang *et al.*, 2016).

It was reported in a study by Rashid *et al.*, (2016) that, air currents from the floor, human movements over the floor and other factors that aerosolize or provide an airborne opportunity for the organism may occur, thus causing human infections through inhalation, horizontal or cross-contamination from other persons, clothing or equipment that the organism resettles upon. Therefore due to the existence of these microbiological pathogens on shoe soles rapid spread of microorganisms in the indoor environment can be directly related to the organisms on floors getting picked up and carried by shoe soles and retransferred to floors in other areas by human movement. *Staphylococcus aureus* had the highest percentage prevalence (45.6 %) this is because it is commonly found on the surface of human skin and is easily transferred to high hand-touch areas such as walls, door knobs, desks, seats etc. The percentage prevalence of S. aureus isolates observed in this study was higher than that reported by Anyanwu and Awa-Agwu (2011) but lower than that observed in a study by Akinrotoye *et al.*, (2019) where 50.6 % *S. aureus* was isolated from fomites in community schools within Abeokuta environs in Nigeria. In this study *Streptococci spp* had a prevalence of 29.8 %, they are members of the normal microbial flora of animals and humans and some can cause diseases that range from sub-acute to acute and even chronic disease such as glomerulonephritis, pneumococcal pneumonia, scarlet fever (Patterson, 1996). Streptococci are also said to be indicators of pollution. Coagulase negative Staphylococci had the least prevalence of 24.6 %, CoNS for example *S. epidermidis* are common human skin commensals although some species can cause infections (Foster, 1996). The indoor air of the lecture theatres were highly polluted with *S. aureus*. The quality of air in offices, lecture theatres, private and public buildings is an important determinant of people's healthy life and wellbeing since many people spend 24-30 % of their day in school buildings or offices. Therefore healthy and safe environment is needed to thrive, learn and succeed (WHO, 2010, Samson *et al.*, 2017). The isolation of S. aureus from indoor of the lecture theatres corresponds with previous studies (Zewudu *et al.*, 2019, Kumari *et al.*, 2015, Kavita and Jyoti, 2012). Short-term inhalation of *S. aureus* can result in the nasal carriage; inhalation or exhalation and contact with the surfaces might be a possible means of transmitting resistant *S. aureus* (Augustine *et al.*, 2017). Antibiotics susceptibility test of the *S. aureus* isolates showed that Chloramphenicol (73.1 %) had the greatest activity followed by gentamicin (69.2 %). Amoxicillin (a beta lactam) was the least active (100 % resistance) followed by erythromycin (80.8 %) and

trimethoprim (65.4 %). The different mechanisms of action of these antibiotics may be responsible for these antibiotics susceptibility profile of the *S. aureus* isolates observed. Chloramphenicol inhibits protein synthesis, it binds to the 23S ribosomal RNA on the 50S subunit of bacterial ribosome and inhibits the action of peptidyl transferase enzyme. In general most S. aureus isolates are resistant to beta lactam antibiotics due to beta lactamase production (Foster, 2017). The antibiotics susceptibility observed with *S. aureus* in this study is similar to that reported by Akinrotoye *et al.*, (2019). High percentage of multidrug resistance (73.1 %) and 19.2 % extended drug resistance was observed. This was an indication that there is a possibility of antibiotics abuse among the students and lecturers using the lecture theatres. All the S. aureus isolates except one had MAR index greater than 0.2, this indicates that the isolates originated from an environment where antibiotics are misused. This is actually alarming because these isolates has tendency to cause infections especially when in contact with immunodeficient or immunocompromised individuals. With the high level of multidrug resistance in this study such infection will be difficult to treat. Proper sanitation of the lecture theatres is therefore recommended in order to prevent the spread of infection through the air and the surfaces. These measures include mopping the floors at least every day with disinfectants, dusting the desk and seats daily before the activities of the day kicks off and sweeping the lecture halls every day at the close of work, dusting the ceiling and standing fans at least twice a month, cleaning the ceiling by removing cobwebs at least twice a week and other sanitary measures which the class may deem fit.

CONCLUSION:

There was a high prevalence of multidrug resistant *S. aureus* in the lecture theatres of Faculty of Pharmaceutical Sciences, Ahmadu Bello University. More awareness about the effects of abuse and misuse of antibiotics should be created among the faculty students and also the need for proper cleanliness of the lecture theatres.

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