PLASMID MEDIATED AMPICILLIN RESISTANT BACTERIAL ISOLATES FROM UNIVERSITY OF ILORIN HEALTH CENTRE

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ABSTRACT: A study on plasmid mediated Ampicillin resistant bacterial isolates from University of Ilorin Health Services Department was carried out. It showed that the Health Service Department environment comprises a mixture of bacteria that are resistant and susceptible to Ampicillin. Most of the isolates are Hospital Acquired Pathogens which cause nosocomial infections. The method of collection of the isolates includes swabbing (desk, bed and sink) with swab sticks and exposure of plates to air. They were analysed using standard bacteriological methods. It showed that – laundry section floor (LSF) has the highest count (7.80 x 10^3 Cfu/ml) while the injection room desk (IRD) had the lowest count (1.00 x 10^1 Cfu/ml). The organisms isolated from various sites include: *Bacillus megaterium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter* spp*, Streptococcus pyogenes, Enterobacter aerogene, Alkaligenes feacalis, Chromobacterium* spp, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The isolates were tested for susceptibility using well diffusion method and confirmed by MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) test respectively. The result showed that 3 of the isolates were susceptible to the antibiotic while 7 were resistant. It showed that *Bacillus megaterium* and *Klebsiella pneumoniae* were susceptible to the different concentration of ampicillin. *Chromobium* spp., *Alkaligenes feacalis*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. were resistant to the different concentrations of ampicillin used. *Streptococcus pyogenes* and *Staphylococcus epidermidis* were only susceptible at ampicillin concentration of 120 µg/ml of ampicillin and resistant to other concentrations. *Staphylococcus aureus* was resistant at ampicillin concentration of 30µg /ml and 60µg/ml and susceptible at ampicillin concentration of 90µg/ml and 120µg/ml. After subjecting the antibiotics to all the isolates, the susceptible ones were confirmed by checking for the MIC. All the three isolates show clarity at concentration of 7.50µg/ml and 3.75µg/ml respectively. The minimum bactericidal concentration (MBC) showed that there was a growth of *Bacillus megaterium* at concentration of 3.75µg/ml of ampicillin, *Staphylococcus aureus* grew at the concentration of 30µg/ml and 15µg/ml while there was growth of *Klebsiella pneumoniae* at the concentration of 30µg/ml. The resistant isolates were cured with plasmid curing method using acridine orange as an agent of knocking out bacteria with R plasmid. Four (4) were susceptible and 3 were resistant after the plasmid curing. It showed that *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were susceptible at 40µg/ml, 20µg/ml and 11µg/ml concentrations of ampicillin after plasmid curing while *Alkaligenes feacalis*, *Enterobacter aerogenes* and *Acinetobacter* spp were resistant at the same concentrations after plasmid curing.

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1. INTRODUCTION

The prevalence of antimicrobial resistant bacteria in health centre is the prime cause of infection that defies normal antimicrobial therapy. Usually, the infections that are often encountered in health centres are caused by microorganisms present in the same environment. A health centre can be described as an environment where group of Doctors see their patients and where some local medical services have their offices (Nester *et al*., 2004).

Nosocomial infections are infections which are as a result of treatment in a hospital or a health care service unit. Infections are considered

nosocomial if they first appear 48 hours or more after hospital admission or within 30days after discharge (Burke, 2009). Nosocomial infections are transmitted due to the fact that hospital officials become complacent and do not practice correct hygiene on regular basis. Also, increase uses of out-patient treatment, i.e. people who are hospitalized are more ill and have more weakened immune systems that have been true in the past. Nosocomial infection can also be referred to as Health Centre Infection or Hospital Acquired Infection (HAI) (Steed, 1999). Moreover, some medical procedures by pass the body's natural protective barriers. Since medical staffs move from patient to patient, they serve as a means for spreading the pathogen (Weese *et al*., 2007).

An epidemiology study of nosocomial infections which developed after patient's admission in Lagos-state University Teaching Hospital (LUTH) from January 2004 to June 2005, has presented 1081 nosocomial infections were reported in 932 patients, (484 males and 448 females) during the 18months period was 14,428 (9196males and 5232 females). An overall infection, 42% came from surgical, 20% from paediatric, 18% from medical and 20% from obstetrics ward. Surgical wounds were the most frequently reported site of infection accounting for 35% and other sites affected were urinary tracts 28% of the 17% which occurred in other systems, 3% was due to a primary bacteraemia (Daniel, 2007). Patients who acquire nosocomial infections seem to remain in the hospital longer than patients without such infections. However, length of stay in hospitals is related to variables such as primary discharge diagnosis, operative procedures and age of the patient; patient with nosocomial infections have been shown to differ from uninfected patients with respect to these same variables (Jonathan *et al*., 2008). Primary disease, operation and age must be controlled in the analysis if these other variable on length of hospital stay is to be separated from it effects (Jonathan *et al*., 2008).

Despite the introduction of many effective antibiotics and an increased understanding of appropriate infection control measures, new pathogens continue to emerge as nosocomial opportunistic. Some of these bacteria have developed increased antibiotic resistance. Others have acquired the ability to survive in antiseptics, intravenous, infusion solutions or on intravascular catheters and still others are ubiquitous in the hospital and readily colonize immunocompromised patients. Virtually any organism can become a nosocomial pathogen (Kenneth and Stephen, 2005). For examples, anaerobic gram positive bacteria, *Clostridium perfringes* which cause gas gangrene, Staphylococci which are usually found in the hospital environment, some types are commensal on skin, they may be found in the nasopharynx (portion of the pharynx – cavity at the back of the mouth above the soft palate), axillae (armpit) and perineum (wedge shaped structure situated between the rectum and the external genitalia) of some individuals. They cause infection that include boils, impetigo (skin inflammation), wound infection, endocarditis (inflammation of the heart), pneumonia, osteomylitis (inflammation of the bone marrow), toxic shock syndrome (complication arising from use of tampon) and septicaemia (multiplication of living bacteria in blood steam causing infection).

Some genus produces the enzyme coagulase, some produces the exotoxins and some are methicillin resistant (Kenneth and Stephen, 2005). Gram negative Enterobacteriaceae such as *Klebsiella* species, *Proteus* species, *Escherichia coli, Enterobacter aerogene.* The source of organisms that cause nosocomial infection may be either endogenous or exogenous to the patient. Endogenous infections are caused by patient own normal flora e.g. *Staphylococcus aureus*, *Escherichia coli*. *Staphylococcus* from the bowel may infect wound following intra-abdominal surgery, also dental caries and periodontal disease caused by normal flora of the oral cavity. Exogenous infections result from transmission infection other than the patient (Steed, 1999). They are referred as opportunistic pathogens.

Plasmids are small, double stranded deoxyribonucleic (DNA) molecule that can exist independently of the chromosome; is also an extra chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently from the chromosomal DNA. Plasmids occur naturally in bacteria, but are sometimes found in eukaryotic organism (Lipps, 2008). Plasmids are able to replicate autonomously, single copy plasmid produce one copy per host cell (Prescott *et al*., 2007). Some plasmids include an additional system or post segregational killing system (PSK), such as host killing or suppressor of killing system of plasmid R1 in *E coli*. They produce both a long- lived posion and a short lived antidote. Daughter cells that retain a copy of the plasmid survive, while a daughter cell that fails to inherit the plasmid dies or suffers growth rate because of the lingering poison from parent cell (Kandavelou and Chandrasegran, 2008).

Ampicillin is a beta-lactamase antibiotic, a part of the amino- penicillin family, they are amino acids and peptide antibiotics. Ampicillin has a broad spectrum activity. i.e. it demonstrates activity for Gram positive organisms such as *Staphylococci* and *Streptococci* and Gram- negative organisms such as *Haemophilus influenza*, *Coliforms*, *Proteus* species, *Salmonella* and *Shigella*. Ampicillin is a derivative of 6-aminopenicillanic acid, stable in acid medium. Ampicillin can interact with other drugs and its effective of Ampicillin may be impacted (Britta, 1997). Ampicillin can be administered orally (liquid or capsules), it can also be injected (Nester *et al*., 2009). It can sometimes result in reactions that range in severity from a rash to potentially lethal allergic reactions such as anaphylaxis. Ampicillin is relatively non- toxic and adverse effects of a serious nature are encountered only infrequently. Resistance to Ampicillin by the targeted bacteria is a result of the possession of one or more resistance gene. Such genes are called resistance plasmid. Ampicillin is often used as a selective agent, to select for and to confirm the uptake of genes. Bacteria possessing such resistant gene are grown in a medium containing Ampicillin and an exchange process such as conjugation, transduction and transformation is done. Only the bacteria that successfully take up the desired genes become Ampicillin resistant and therefore, contain the other desired gene as well. A single plasmid may carry genes for resistance to several drugs; pathogen population may become resistant to several antibiotics simultaneously even if the patient is being treated with only one drug (Jawetz *et al*., 1995).

The aim of this study is to isolate bacterial pathogens, test the effectiveness of the antibiotics (Ampicillin) on susceptible organisms as well as highlight factors (plasmid) relating to resistant of an organism against the antibiotics (Ampicillin).

2. MATERIALS AND METHODS

2.1. COLLECTION OF SAMPLES

The samples were collected from University of Ilorin Health Centre (Clinic) permanent site. The samples were obtained from various sections of the clinic including: Female ward (floor, air and bed), Consulting room (air), Record section (air), Pharmacy section (NHIS desk, student desk, and sink), Laboratory section (floor and air) and General toilet (floor and air). Nutrient Agar (NA) was exposed in different places for 30seconds. After which they were collected and incubated at 37ºC for 24 hours. The colony developed after 24hours of aerobic incubation. The colonies were counted and recorded. Swabbing method was also used for the floor, sink, desk and bed. In this method of sample collection, a square meter portion was divided into 8 divisions and a swab of one of the divisions was made carefully after moisturizing the swab stick with normal saline (NaCl). The swabs were dissolved in 9ml of sterile distilled water a 1:10 dilution; it was then plated on Nutrient Agar at 37C for 18-24hours. The colony developed was counted and multiplied with dilution factor to determine the colony count. All the colonies obtained were then sub cultured into a pure culture.

2.2. ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF ISOLATES

Media used for this study were prepared according to manufacturer's instruction and after sterilized by autoclaving at 121ºC for 15minutes. Different colonies observed on the pure culture were used for identification and characterization also the pure cultures were put into the McCarthy bottle containing slant of nutrient agar at 45º degree as sock in the refrigerator at 7ºC for subsequent use. Pure cultures of all the organisms were subjected to different biochemical tests for proper identification and characterization of the organisms (Bergey's Manual of Determinative Bacteriology, Buchanan and Gibbons, 1981; Fawole and Oso, 2004).

2.3. STANDARDIZATION OF INOCULUM

Standardization of inoculum was done by picking about 4-5colonies from plate, transferring it into 9ml of normal saline, the turbidity was observed as more organism were added and was compared with Mc Farlands which are used to reference the turbidity of bacterial suspensions so that the numbers of bacteria will be within a given range. Mc Farlands standard is a mixture of $Bacl_2$ and concentrated HCl (hydrogen chloride) mixing precipitate. To get ranges of Mc Farlands standard, different ml of $BaCL_2$ was mixed with different ml of HCl. i.e. 0.1ml of $BaCl_2$ mix with 9.9ml of HCl gives approximately 3.0 x 10^8 CFU/ml etc. (Palomino and Martin, 2009).

2.4. STANDARDIZATION OF ANTIBIOTICS

Antibiotic used is Ampicillin (250mg). 250mg of Ampicillin was dissolved in a phosphate buffer solution (2%) v/v. The solution was then transferred into a conical flask and made up to 100ml of distilled water in a standard flask then the solution makes a homogenous mixture. Several test tubes were labelled with desired

concentration which are 30µg/ml, 60µgml, 90µg/ml and 120µg/ml according to the concentrations of antibiotics 0.12ml, 0.24ml, 0.36ml and 0.48ml of the homogenous mixture were dispensed into the tubes respectively and was made up to make 10ml with distilled water (Palomino and Martin, 2009).

2.5. ANTIOBIOTICS SUSCEPTIBILITY (SENSITIVITY) TEST FOR THE ISOLATES

The well diffusion method was used. A solidified agar seeded with the organism was used, the organism was emulsified with normal saline, poured on Nutrient Agar, swirled gently and then the excess was drained into a container containing disinfectants. The plates were left to dry.After drying, a sterile cork borer was used to bore holes on the agar, according to the numbers of different concentration desired to be used. The plugged agars from the plates were discarded into the disinfectant container. The wells created were well spaced to prevent clumsiness. The antibiotic prepared with different volumes at different concentrations was filled into the wells without allowing overflow. The plates were covered, not inverted and placed in the incubator for 24 hours at 37ºC. The plates were observed for radial zones of inhibition surrounding the wells (National Committee for Clinical Laboratory Standards, 1999).

2.6. MINIMUM INHIBITORY CONCENTRATION (MIC)

The broth dilution was used. In this technique several dilution of antibiotic in liquid medium was challenged with the standard inoculum of the test organisms. In this test, 2ml of Nutrient broth (double strength) was measured each into several test tubes and sterilized in the autoclave at 121ºC for 15 minutes. Different concentrations of the antibiotics were prepared in different test-tubes and labelled accordingly. Standard inoculums of the test organisms were also prepared in different test tubes. One (1) ml of the standard inoculum and 1ml of the antibiotic concentrations were inoculated into the test tubes containing the nutrient broth. The test tubes were then incubated at 37ºC for 18-24 hours and observed for clarity. MIC test was carried out on organisms that were susceptible to the antibiotic used. The clarity of the result of MIC confirms the susceptibility to the antibiotic. Control methods were also carried out in which the dilution of the antibiotic to serve as turbidity standard (French, 2006). Mathematically, MIC which is the value of the lowest concentration of the antibiotic that will inhibit the growth of the organisms. The desired concentrations were 30µg/ml, 15µg/ml, 7.5µg/ml and 3.75µg/ml.

2.7. MINIMUM BACTERICIDAL CONCENTRATION (MBC)

Determining the Minimum Bactericidal Concentration was done by firstly determining the Minimum Inhibitory Concentration. Minimum Bactericidal Concentration is the lowest concentration of the antibiotics that kills at least 99.9% of the test organism. It was done by taking samples from test tubes prepared for MIC which was different concentration, plated on a Nutrient Agar then incubated at 37ºC for 24 hours. The plates were checked after 24 hours, the lowest concentration of the antibiotic that inhibited the growth was less than 0.1% of the organism, which was taken to be the Minimum Bactericidal Concentration value of the antibiotics (French, 2006).

2.8. PLASMID CURING

The test for the presence of plasmid as resistant determining factor was carried out by using Sub Minimum Inhibitory Concentration of acridine orange. 0.12g of acridine orange was weighed and dissolved in 300ml of distilled water in a standard flask. Then an overnight incubation i.e. 24 hours inoculum was emulsified with Normal saline. 2ml of Nutrient Broth (double strength) was autoclaved for sterility. From the stock solution prepared, the desired concentration of the acridine orange was used to calculate the volume needed and the corresponding water to be used (Peter *et al*., 1989). Since the procedure is a sub minimum inhibitory concentration, prepared test tubes containing 2ml of Nutrient Broth + volume of acridine orange needed + 0.1ml of the inoculum) are used. From test tube A, 1ml was transferred to test tube B and from B 1ml was transferred to test tube C, hence reducing the dilution. It can be drawn that test tube C has less number of organisms, less volume of acridine orange and more volume of Nutrient broth. The volumes needed were put into test tubes and filled with corresponding volume of water to make 10ml. The principle behind this test to use the acridine orange to knock out plasmid factor responsible for the resistance of the isolate (Peter *et al*., 1989).

3. RESULTS ANALYSIS

A study was carried out on plasmid mediated Ampicillin resistant bacterial isolates from University of Ilorin Health Services

Department. Table 1 shows the total viable counts (CFU/ml) of the samples collected. It showed that – laundry section floor (LSF) has the highest count (7.80 x 10^3 Cfu/ml) while the injection room desk (IRD) had the lowest count (1.00 x 10^1 Cfu/ml).

Table 1: Total viable count (CFU/ml)

SITES	Total Viable count (CFU/ML)
FWA – FEMALE WARD AIR	2.00 x 10^1
LS – LABORATORY SINK	5.20 x 10^2
RSD – RECORD SECTION	1.43 x 10^1
LA – LABORATORY AIR	2.20 x 10^2
IRD – INJECTION ROOM DESK	1.00 x 10^1
GTF- GENERAL TOILET FLOOR	6.00 x 10^1
LF- LABORATORY FLOOR	3.00 x 10^1
FWF- FEMALE WARD FLOOR	7.50 x 10^2
MW –MALE WARD	4.20 x 10^2
LSF – LAUNDARY SECTION FLOOR	7.80 x 10^3

Table 2 shows the distribution of bacterial isolates in University of Ilorin health center. It showed that only *Bacillus megaterium* was only present in the air samples taken the health center. *Chromobacterium* spp. was present in the surface of the sinks at the health center. *Staphylococcus aureus* and *Acinetobacter* spp. were present at both the floors and beds. *Klebsiella pneumoniae* and *Staphylococcus epidermidis* were present at both the air and floor samples. *Streptococcus pyogenes* and *Pseudomonas aeruginosa* were present at both floor and sink. *Enterobacter aerogenes* were present at all samples except for air and beds. *Alkaligenes feacalis* was present on all samples except that of desk and sink (Table 2).

Table 2: Distribution of Bacterial Isolates in University of Ilorin Health Center

Isolates	Air	Floor	Bed	Desk	Sink
Bacillus megaterium	+	-	-	-	-
Staphylococcus aureus	-	+	+	-	-
Klebsiella pneumoniae	+	+	-	-	-
Staphylococcus epidermidis	+	+	-	-	-
Streptococcus pyogenes	-	+	-	-	+
Enterobacter aerogenes	-	+	-	+	+
Chromobacterium spp.	-	-	-	-	+
Pseudomonas aeruginosa	-	+	-	-	+
Acinetobacter spp.	-	+	+	-	-
Alkaligenes feacalis	+	+	+	-	-

KEYS: += PRESENT; - = ABSENT

Table 3 shows the antibiotic susceptibility/resistance pattern of bacterial isolates of university clinic at different concentrations. It showed that *Bacillus megaterium* and *Klebsiella pneumoniae* were susceptible to the different concentration of ampicillin. *Chromobium* spp., *Alkaligenes feacalis*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. were resistant to the different concentrations of ampicillin used. *Streptococcus pyogenes* and *Staphylococcus epidermidis* were only susceptible at ampicillin concentration of 120 µg/ml of ampicillin and resistant to other concentrations. *Staphylococcus aureus* was resistant at ampicillin concentration of 30µg /ml and 60µg/ml and susceptible at ampicillin concentration of 90µg/ml and 120µg/ml (Table 3).

Table 3: Antibiotic Susceptibility / Resistance Pattern Of Bacterial Isolates Of University Clinic At Different Concentrations

ISOLATES	ANTIBIOTIC CONCENTRATIONS			
	30µg /ml	60µg/ml	90µg/ml	120µg/ml
B. megaterium	S	S	S	S
E. aerogenes	R	R	R	R
Chromobium spp	R	R	R	R
A. feacalis	R	R	R	R
P. aeruginosa	R	R	R	R
K. pneumoniae	S	S	S	S
Str. pyogenes	R	R	R	S_S
S. epidermidis	R	R	R	S_S
Acinetobacter spp	R	R	R	R
S. aureus	R	R	S	S

KEYS: R – RESISTANT; S – SUSCEPTIBLE; S_S – SLIGHTLY SUSCEPTIBLE

Table 4 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). After subjecting the antibiotics to all the isolates, the susceptible ones were confirmed by checking for the MIC. All the three isolates show clarity at concentration of 7.50µg/ml and 3.75µg/ml respectively (Table 4). The minimum bactericidal concentration (MBC) showed that there was a growth of *Bacillus megaterium* at concentration of 3.75µg/ml of ampicillin, *Staphylococcus aureus* grew at the concentration of 30µg/ml and 15µg/ml while there was growth of *Klebsiella pneumoniae* at the concentration of 30µg/ml (Table 4).

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum Inhibitory Concentration (MIC)				
Isolates	30µg/ml	15µg/ml	7.50µg/ml	3.75µg/ml
B. megaterium	S_T	C	C	C
S. aureus	T	S_T	C	C
K. pneumoniae	C	C	C	C
Minimum Bactericidal Concentration (MBC)				
Isolates	30µg/ml	15µg/ml	7.50µg/ml	3.75µg/ml
B. megaterium	NG	NG	NG	G
S. aureus	G	G	NG	NG
K. pneumoniae	G	NG	NG	NG

Keys: NG = No Growth, G = Growth; S_T – Slightly Turbid; T - Turbid; C - Clarity

Table 5 shows the resistant and susceptible isolates after plasmid curing at different concentrations of amipicillin as well as their MICs values. It showed that *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were susceptible at 40µg/ml, 20µg/ml and 11µg/ml concentrations of ampicillin after plasmid curing while *Alkaligenes feacalis*, *Enterobacter aerogenes* and *Acinetobacter* spp were resistant at the same concentrations after plasmid curing (Table 5).

Table 5: Resistant and Susceptible Isolates after Plasmid Curing at Different Concentrations

Isolates	**Concentrations**			**Minimum Inhibitory Concentration**		
	40µg/ml	20µg/ml	11µg/ml	40µg/ml	20µg/ml	11µg/ml
Pseudomonas aeruginosa	S	S	S	C	C	C
Alkaligenes feacalis	R	R	R	ND	ND	ND
Chromobacterium spp	S	S	R	C	C	T
Enterobacter aerogenes	R	R	R	ND	ND	ND
Staphylococcus epidermidis	S	S	S_S	C	C	C
Streptococcus pyogenes	S	S	S_S	C	C	S_T
Acinetobacter spp	R	R	R	ND	ND	ND

Keys: R = Resistant; S = Susceptible; S_S = Slightly Susceptible; ND= Not Determined; C – Clarity; T – Turbid; S_T - Slightly Turbid

4. DISCUSSION

The results show that the isolates from University of Ilorin Health Centre are mostly resistant to Ampicillin. The floor has the highest number of microbial loads compared to air and sink. The higher microbial load on the floor than air may be as a result of unstable nature of the microflora of air (transient and variable). The numbers and types of microbes present in the air at any time are influenced by activities in that environment and the amount of dust particle present in air (Prescott *et al*, 2007). Furthermore, laboratory, laundry, and general toilet also recorded high microbial load probably as a result of the type of activities common there. Improper usage of toilet, accidents with hypodermic syringes such as self inoculation, spilling of injection content, laboratory scientist carrying out laboratory work without taken to its precaution (Willey *et al*., 2008).

Bacillus spp are present everywhere. It could exist as spore and can survive in the environment for a period of time (Jawetz *et al*., 2004). *Bacillus* spp are highly susceptible to antibiotics. It was observed that Ampicillin used as antibiotic on *Bacillus* inhibited its growth as evidenced by the zones of inhibition.

Klebsiella pneumoniae cause a classic form of primary pneumonia. It can also cause nosocomial infections and it is among the eight (8) most important nosocomial pathogens in hospital (Duguid, 1989). *Klebsiella pneumoniae* is known to be sensitive to Ampicillin - a broad spectrum antibiotic (Mordi and Moses, 2008). Klebsiella pneumoniae was found to be susceptible to Ampicillin at different concentrations indicating that Ampicillin is still effective against this organism.

Enterobacter aerogenes has the general characteristics of *Klebsiella* sp. but can be differentiated by being motile and ornithine positive. It is frequently encountered in clinical specimen. It is widely distributed in water, sewage, soil and vegetable. The organism is associated with a variety of infection involving respiratory tract, cutaneous wound and occasionally causing septicaemia hence it needs to be controlled (Parodi *et al*., 2008). *E. aerogenes* from the results obtained was resistant to Ampicillin, even after curing with acridine orange.

Staphylococcus spp. is the predominant isolates found in this study. *S. aureus* are ubiquitous, it is also a normal flora of the skin yet it can cause various kinds of infection especially

when there are cases of indiscriminate use of antibiotics or overgrowth of normal flora (Tuo *et al*., 1995). It has also been reported to be one of the commonest causes of wound infections, burns and the commonest gram positive microorganism causing infection (Duguid, 1989). *S. aureus* and *S. epidermidis* were relatively susceptible to Ampicillin. It was also susceptible at a higher concentration after plasmid curing. *Streptococcus pyogenes* is considered aerotolerant, however some strains are anaerobic. Most Streptococci are parasites of humans and animals (Pelczar *et al*., 2007). It was observed that the gram positive coccus was susceptible to Ampicillin at a high concentration.

Resistance of Isolates to Ampicillin even after the plasmid curing might be attributed to environmental factors, such as indiscriminate use of antibiotics, emergence of new strains of the organisms, activities of Medical personnel to patients, methods of good hygiene in the Hospital environment as related to disinfecting and cleanliness (Jacob *et al*., 1991).

5. CONCLUSION

Majority of the organisms isolated from the hospital environment are saprophytes that cause infection. To prevent this, Hospital environment should be well disinfected, equipment should be properly sterilized before use. Lastly, research should be intensified in the area of chemotherapeutic agents; more effective antibiotic should be discovered that can be effective against organisms with resistant plasmid.

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