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Microorganisms Associated With Usable Equipment in The Radiological Unit of University of Benin Teaching Hospital

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ABSTRACT: Radiology unit is one of the commonly used diagnostic centres in the hospital. Medical devices in this unit harbour nosocomial pathogens that may likely complicate patient illnesses. This study investigated the presence of pathogenic microbes in usable equipment from hospital radiology units. Samples were collected via sterile swab stick from three radiological units (Ultrasound, X-ray and Mammogram rooms) equipment of the University of Benin Teaching Hospital, Benin City. The media used in the isolation of the microorganisms were Nutrient Agar (bacteria) and Potato Dextrose Agar (fungi). The highest total heterotrophic bacterial count (THBC) was found to be $3.33 \pm 0.33 \times 10^2$cfu/cm^2 from X-ray cassette while the least $1.00 \pm 0.33 \times 10^2$cfu/cm^{2}was from Mammography machine. Total heterotrophic fungi (THFC) showed the highest count of $1.33 \pm 0.33 \times 10^2$ cfu/ cm^2 while the least $2.70 \times 10^1 \pm 0.33$ cfu/cm^{2}were from X-ray cassette and Mammogram (compressor) respectively.Bacterial identified based on cultural, morphological and biochemical characteristics were *Micrococcus luteus, Escherichia coli, Staphylococcus epidermidis, Bacillus polymyxa, Corynebacteriumkutsceri, Streptococcus pneumoniae, Bacillus subtilis, Enterococcus faecium, Streptococcus mitis, Bacillus cereus, Staphylococcus aureus* and *Pseudomonas aeruginosa. Staphylococcus aureus* (17.24 %) was the most occurring bacterial isolate while the least were *M. luteus* and *S. mitis* with 3.45 % occurrence. Fungi isolated include *Penicillium* sp., *Fusarium* sp. and *Mucor*sp.*Pseudomonas aeruginosa, E. coli, S. mitis* and *M. luteus*were more susceptible to all the conventional antibiotics while *B. subtilis,B. cereus* and *S. aureus*showed more resistance to some of the antibiotics. The risks of contracting a nosocomial infection due to the presence of microorganisms isolated in this study is very high, therefore, the control of microorganisms is of prime importance in hospital environments.

Keywords: Microorganisms, Equipment, Radiology, Hospital

Introduction

Diagnostic equipment are located and used in outpatient unit centres in the hospitals. These diagnostic tools are employed to provide useful information about the health of an individual, risk assessment, checking the course of an infection, monitoring a patient's treatment response to provide a guide for further test and treatment (Odonkor *et al*., 2015). One of the most commonly used diagnostic centre is the radiology centre. The centre plays a useful role in medical diagnosis (Ochie and Ohagwu, 2009). Various subunits such as Ultrasound, X-ray and Mammogram units are found in the radiology centres and the most commonly used tool is the X-ray machine.

The role of these medical devices, such as bronchoscopes, in the transmission of healthcare-associated infections (HAIs) has long been recognized, however, the evidence that environmental and medical equipment surfaces play a role in the transmission of HAIs has been weak (Oshoma *et al*., 2010). Studies have demonstrated that pathogens

can be transmitted from surfaces to personnel and patients, and that these pathogens are not adequately removed by routine room cleaning. This has led to an increased focus on the importance of cleaning and disinfecting hospital surfaces and medical equipment and efforts to assess and improve the effectiveness of these practices (Eze *et al*., 2013).

Nosocomial infection also known as hospital acquired infection is infection acquired in a hospital environment, which was not present in the patient at the time of admission (Velvizhi and Sucilathangam, 2013). Hospitals are potentially conducive for antimicrobial resistant and virulent pathogens to proliferate. Large numbers of microorganisms are found in hospital equipment and it is of great importance to carry out regular survey as a yardstick of determining standard of cleanliness in hospitals (Williams *et al*., 2006; Velvizhi and Sucilathangam, 2013). It has also been reported that poor handling and maintenance of hospital equipment have played significant role in the spread of microorganisms in hospital environments(Eze *et al*., 2013).

It is a known fact that radiology centre in most hospital have a high influx of both outpatient and inpatient, therefore, standard procedure and control guidelines should be put in place to prevent transmission of pathogenic microorganisms. Microorganisms have been reported to contaminate radiological equipment (Ohara *et al*., 1999). These have led to proliferation of microorganisms and resulted in infectious diseases. This scenario could have important implications for public health and infection control, but to date the scientific literatures have dealt little with matters relating to microbiological monitoring in radiological equipment (Odonkor *et al*., 2015). Healthcare–associated infections are a cogent issue for the radiological equipment and knowledge of how to prevent them is increasingly required by health professionals. The aim of thisstudy was to investigate the presence of pathogenic microbes in usable equipment from hospital radiology units.

Materials and Methods

***Sample collection*:** Samples were collected directly with sterile swab stick from three radiological units (Ultrasound, X-ray and Mammography rooms) equipment of the University of Benin Teaching Hospital, Benin City and were taken immediately to the laboratory for further analysis.

Enumeration of Microorganisms: The swab sticks were dipped into 10 ml of sterile distilled water, vortexed and allowed to stand for 10 min. From this stock, 10-fold serial dilution was carried out in cleaned sterile test tubes containing 9 ml of sterile distilled water. From the aliquots, 0.1 ml was transferred into Nutrient agar (NA) and 0.1 ml was transferred into Potato dextrose agar (PDA) plates. Plates containing NA were incubated at 37 ^{o}C for 48 h and colonies counted while PDA plates were incubated at 28±2 ^{o}C for 72 h. The method described by Public Health England (2014) for estimating bacterial and fungal counts was used to enumerate the total viable counts of the isolates. The discrete colonies on the Nutrient agar and Potato dextrose agar were selected and counted. The mean colony count on the nutrient agar and potato dextrose plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per centimeter square (cfu/cm^2).

***Identification of microbial isolates*:** From the Nutrient agar (NA) plates, colonies were randomly picked and repeatedly sub-cultured on Nutrient agar (NA) for purification. Purified bacterial isolates were stored in Nutrient agar (NA) slants for further studies. The purified bacterial isolates were characterized by morphology, Gram's reaction and biochemical test using the scheme in Bergey's manual of determinative bacteriology (Holt *et al*., 1994; Cheesbrough, 2000).

Fungal isolates were examined macroscopically and microscopically using the needle mounts technique. Their identification was performed according to the procedure of Barnett and Hunter (1972) and Larone (1986).

***Antibiotics susceptibility pattern*:** Antimicrobial disc tests of the isolates were performed according to the recommendations of the National Committee Laboratory Standards (NCCLS) (2007) using the following antibiotic discs pefloxacin (10 µg), gentamycin (10 µg), ampiclox (30 µg), zinnacef (20 µg), ciprofloxacin (10 µg), streptomycin(30 µg), septrin (30 µg), erythromycin (10 µg), amoxicillin (30 µg) and rocephin (25 µg). The test organisms were inoculated into sterile nutrient broth in a test tube and incubated at 37 ^{o}C for 24 h. From the liquid culture, 0.1 ml was transferred into solidified Nutrient agar (NA) in a petri-dish and a sterile spreader was used to distribute evenly in the agar. The plates were allowed to dry for 5 min there after the standard antibiotics disc was laid on the inoculated agar. The plates were incubated at 37 ^{o}C for 24 h. Clear zones around the discs were measured

and interpreted as either susceptible or resistant for the test organisms to the particular antibiotic. Zones of inhibition ≥ 13 mm were considered sensitive while those ≤ 12 mm were regarded resistant (NCCLS, 2007).

The multiple antibiotic resistance index (MARI) of bacterial isolated was calculated as the ratio between number of antibiotics for which an isolate is resistant and the total number of antibiotics to which the organism was exposed (Akinjogunla and Enabulele, 2010).

Results

The result of total heterotrophic bacterial count (THBC) and total heterotrophic fungi count (THFC) on hospital equipment is shown in Table 1. The highest THBC was found to be 3.33±0.33 x 10^2 cfu/cm^2 from X-ray cassette while the least 1.00±0.33 x10^2 cfu/$cm^2$was from Mammography machine. THFC showed the highest count of1.33±0.33 x10^2 cfu/ cm^2 while the least 2.70 x10^1±0.33 cfu/cm^2were from X-ray cassette and Mammogram (compressor) respectively.

Table 1: Total heterotrophic bacterial count (THBC) and total heterotrophic fungi count (THFC) on hospital equipments

Equipment	THBC (cfu/ cm^2)	THFC (cfu/cm^2)
Mammography machine	1.00 ± 0.00 x10^2	0.00
Transabdominal probe (R3)	3.00 ± 0.00 x10^2	8.00± 0.20 x10^1
X-ray cassette	3.33± 0.33 x10^2	1.33 ± 0.33 x10^2
Soft tissue probe (R1)	1.00 ± 0.00 x10^2	0.00
Water solvent (processor)	1.00 ± 0.00 x10^2	0.00
X-ray tube (head)	1.00 ± 0.00 x10^2	0.00
Transvaginal probe (R2)	0.00	0.00
Transvaginal probe	1.00 ± 0.00 x10^2	0.00
X-ray processor (fixer)	2.33 ± 0.33 x10^2	7.70± 0.23 x10^1
Developer (processor)	8.00 ± 0.20 x10^2	0.00
X-ray room (table)	2.00 ± 0.00 x10^2	1.00 ± 0.00 x10^2
Soft tissue probe (R3)	0.00	5.70 ± 0.22 x10^1
Transabdominal probe (R2)	0.00	0.00
Processor (body of machine)	1.67 ± 0.33 x10^2	1.00 ± 0.00 x10^2
Mammogram (compressor)	1.00 ± 0.00 x10^2	2.70 ± 0.33 x10^1
Mammogram compressor pad	1.00 ± 0.00 x10^2	0.00

The cultural, morphological and biochemical characteristics of bacterial isolates obtained from the various units are shown in Table 2. Bacteria isolated from various units were *Micrococcus luteus, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus epidermidis, Bacillus polymyxa, Corynebacteriumkutsceri, Streptococcus pneumoniae, Bacillus subtilis, Enterococcus faecium, Streptococcus mitis, Staphylococcus aureus* and *Bacillus cereus*.The frequency of occurrence of bacteria is shown in Table 3. *Staphylococcus aureus* (17.24 %) was the most occurring bacterial isolate while least occurring bacterial isolates were *M. luteus* and *S. mitis* with 3.45 % occurrence respectively.

The cultural and morphological characteristics of fungi isolates identified were *Penicillium* sp., *Fusarium* sp. and *Mucor* sp. as shown in Table 4

The antibiotics susceptibility test of the isolates are shown in Table 5 while the multiple antibiotic resistance (MAR) index as shown in Table 6.*Pseudomonas aeruginosa, E. coli, S. mitis* and *M. luteus*were more susceptible to all the conventional antibiotics with MAR index of 0.40 while *B. subtilis, B. cereus* and *S. aureus* showed more resistance to some of the antibiotics with MAR index of 0.70.

Table 2: Cultural, morphological and biochemical characteristics of bacterial isolates

Test	**Org 1**	**Org 2**	**Org 3**	**Org 4**	**Org 5**	**Org 6**	**Org7**	**Or8**	**Org 9**	**Org 10**	**Org 11**	**Org 12**
Shape	Round	Round	Round	Round	Round	Round	Round	Round	Circular	Round	Circular	Circular
Colour	Cream	Cream	Milky	Cream	Milky	Milky	Creamy	Orange	Orange	Milky	Cream	Milky
Margin	Entire	Entire	Entire	Entire	Entire	Lobate	Entire	Entire	Entire	Serrated	Entire	Entire
Opaque	Opaque	Opaque	Opaque	Translucent	Opaque	Opaque	opaque	opaque	Opaque	Translucent	Opaque	Opaque
Elevation	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Raised	Flat	Flat	Flat
Wet/dry	Wet	Dry	Wet	Wet	Wet	Dry	Wet	Wet	Wet	Wet	Dry	Wet
Gram reaction	+	-	-	+	+	+	+	+	+	+	+	+
Shape	Cocci	Rod	Rod	Cocci	Rod	Rod	Spherical	Bacilli	Spherical	Cocci	Cocci	Rod
Arrangement	Clusters	Single	Single	Clusters	Chains	Chains	single	Single	Single	Chains	Clusters	Single
Catalase	-	+	+	+	+	+	+	+	-+	+	+	+
Oxidase	+	-	+	-	+	-	-	+	+	-	-	-
Indole	-	+	+	-	+	+	-	+	+	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	+	-
Citrate	-	+	+	-	-	-	-	-	-	-	+	-
Coagulase	-	-	-	+	-	-	-	-	-	-	+	-
Spore	-	-	-	-	+	-	-	+	-	-	-	+
Fermentation												
Lactose	+	+	-	+	+	-	+	+	-	+	-	+
Sucrose	+	-	-	-	+	+	+	+	+	-	-	+
Sorbitol	-	-	-	-	-	-	-	+	-	-	+	-
Glucose	-	+	+	+	+	+	+	+	+	+	+	+
Manitol	-	+	-	-	+	+	-	+	+	-	+	-
Possible identity	*M.luteus*	*E. coli*	*P. aeroginosa*	*S. epidermidis*	*B. polymyxa*	*C. kutsceri*	*S. pneumoniae*	*B. subtilis*	*E. faecium*	*S. mitis*	*S. aureus*	*B. cereus*

Org. 1 = *Micrococcus leteus,* Org. 2 = *Escherichia coli,* Org. 3=*Pseudomonas aeroginosa* Org. 4 = *Staphylococcus epidermidis,* Org. 5 = *Bacillus polymyxa,* Org. 6 = *Corynebacteriumkutsceri,* Org. 7 = *Streptococcus pneumoniae,* Org. 8 = *Bacillus subtilis,* Org. 9 =*Enterococcus faecium,* Org. 10 = *Streptococcus mitis,* Org. 11= *Staphylococcu aureus,* Org. 12 = *Bacillus cereus*

Table 3: Percentage frequency of occurrence of bacterial isolates found in the radiological equipment

Microorganisms	**Percentage occurrence (%)**
M. luteus	3.45
E.coli	13.79
P. aeroginosa	13.79
S. epidermidis	6.90
B. polymyxa	10.34
C. kutsceri	6.90
S. pneumoniae	6.90
B. subtilis	3.45
E. faecium	6.90
S. mitis	3.45
S. aureus	17.24
B. cereus	6.90
Total	100

Table 4: Cultural and morphological characteristics of fungi isolates

Isolate	Cultural	Microscopic examination	Fungal isolates
F1	Green flat colony with reverse side dirty white	Brush-like conidia,, septate branching conidiophores was smooth/rough	*Penicillium* sp
F2	White and cottony mycelium	Multi-segmented canoe-like spores with barnached and segmented conidiophores	*Fusarium* sp
F3	Thick-abundant cottony mycelium, and white reverse	Non-septate hyphae with sporangium containing black, sporangiosphores, columella separated by septum	*Mucor* sp

Table 5: Antibiogram of the bacterial isolate in the first and last batch

Microorganisms	APX (30 µg)	Z (20 µg)	AM (30 µg)	R (25 µg)	CPX (10 µg)	S (30 µg)	SXT (30 µg)	E (10 µg)	PEF (10 µg)	CN (10 µg)
Staphylococcus aureus	R	R	R	S	R	S	R	R	S	R
Pseudomonas aeruginosa	S	S	S	R	R	S	R	S	R	S
Escherichia coli	S	S	S	R	S	R	S	S	R	R
Bacillus subtilis	R	R	R	R	S	R	R	S	R	S
Micrococcus luteus	S	S	R	S	S	S	R	R	S	R
Staphylococcus epidermidis	R	R	S	S	S	R	R	S	R	R
Bacillus polymyxa	R	R	R	S	S	R	S	R	S	S
Corynebacteriumkutsceri	S	S	S	R	R	R	S	S	R	R
Streptococcus pneumonia	S	R	S	S	S	R	R	R	S	R
Enterococcus faecium	S	R	S	S	S	R	R	R	S	R
Streptococcus mitis	R	S	S	R	S	S	R	S	S	R
Bacillus cereus	S	R	R	R	R	S	S	R	R	R

Key
PEF: Pefloxacin, CN: Gentamycin, APX= Ampiclox, Z= Zinnacef, CPX= Ciprofloxacin, S= Streptomycin, SXT= Septrin, E= Erythromycin, AM = Amoxicillin, R = Rocephin
S = Susceptible, R = Resistance.

Table 6: Multiple antibiotic resistance profile of bacterial isolates from radiological equipment

Isolates	Multiple antibiotic resistance index
M. luteus	0.40
E.coli	0.40
P.aeroginosa	0.40
S. epidermidis	0.60
B. polymyxa	0.50
C. kutsceri	0.50
S. pneumoniae	0.50
B. subtilis	0.70
E. faecium	0.50
S. mitis	0.40
S.aureus	0.70
B. cereus	0.70

Discussion

The study showed that radiology equipment and accessories are involved as reservoirs of nosocomial microbes. The investigation was aimed at assessing the presence of pathogens associated with radiologicalequipment in the Hospital. The results confirmed that the various units and accessories had some microbial counts considered to be infectious. These nosocomial organisms are harboured by the equipment. Hospital acquired infection has been reported to be on the increase, due to poor hygiene practices, since there is no strict monitoring or control of hygiene level in the Radiology Department (Ochie and Ohagwu, 2009). Cleaning of equipment and accessories after usage with water alone was believed to be an adequate measure, but this can also inoculate radiological equipment with microorganisms. X-ray cassette as found to have the highest microbial count, due to the fact that is the most frequently used tool by the Radiographers. Contamination of equipment maybe due to inappropriate cleaning procedure with disinfectant or decontamination method is faulty and high microbial load might be due to collection time. The contamination level of X-ray equipment observed in this study indicates the level of hygienic practices and length of stay of patients in the hospital. High bacterial counts from various units may be due to direct contact with the skin of patients and health workers during scanning procedures, allowing it to be a potential spread of nosocomial infections (Levin *et al*., 2009; Oshoma *et al*., 2010).

The results of this study identified some bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa Escherichia coli, Bacillus cereus, Streptococcus faecalis Bacillus subtilis, Micrococcus leteus, Staphylococcus epidermidis, Bacillus polymyxa, Corynebacteriumkutsceri, Streptococcus pnuemoniae, Enterococcus faecium,*and *Streptococcus mitis* from all the various units. In a related study, *Klebsiella*spp, coliform,*Staphylococcus aureus* and coagulase negative *Staphylococcus epidermidis*were identified on X-ray equipment and accessories (Ochie and Ohagulu, 2009). These bacteria are potential nosocomial pathogens not only due to their prevalence but can also invade the body through wound or any open route. Prevalence of *Staphylococcus aureus*was high, reasons beeing that the bacterium is a normal flora of the skin. It causes a lot of illnesses ranging from pimples, impetigo, cellulitis, scalded skin syndrome, abscesesetc to life threatening sicknesses like pneumonia and meningitis. It is still a major nosocomial infection (Nester *et al*., 2004).

Pseudomonas aeruginosa is often isolated in water and damp areas. *Pseudomonas aeruginosa* infection may be mild as in external otitis and severe in wound infections. However, high morbidity rate of infection is associated with immuno-compromised individual or health challenges such as diabetes (Eze *et al*., 2013). Interestingly, same group of bacterial genera have been implicated as air micro flora of X-ray room from private hospital, mostly

Staphylococcus and *Pseudomonas* spp (Oshoma *et al*., 2010). *B. cereus*, *B. subtilis* and *C. flavescens* from X-ray equipment used in the hospital could be as a result of inadequate decontamination of the surfaces in these hospitals (Akindele *et al*., 2010).

The prevalence of these pathogens is lower than the earlier prevalence rate of *S. aureus* (30.2 %) and *P. aeruginosa* (12.0 %) as reported by Burge *et al*. (2000) from some hospital equipment. The prevalence of the pathogens in this work is higher than the earlier work reported by Dancer (2008) that *S. aureus* (6.7 %) and *P. aeruginosa* (5.2 %) respectively. The study confirmed the report of Dancer (2008), Boone and Gerba (2007), and Ohara *et al*. (1999) that *S. aureus* and *P. aeruginosa* are the major contaminants of hospital equipment such as blood pressure cuffs, stethoscopes, pulse oximetry sensors, ultrasound transducers and telephones. The high level of contamination of these pathogens could also be as a result of inadequate decontamination of the microbial load from the surfaces (Addy *et al*., 2004)

This finding corroborates earlier report of Fekety *et al*. (2001) that surfaces can act as reservoirs of microbes which could in turn lead to the spread of infection upon being touched, by either healthcare workers, patients or visitors. Crowded conditions within the hospital, frequent transfer of patients from one unit to another, and concentration of patients highly susceptible to infection in one area such as newborn infants, burn patients, and intensive care, may contribute to development of nosocomial infections due to contaminated surfaces. Microbial flora may contaminate surfaces of objects, devices and materials which subsequently contact susceptible body sites of patients (Jawad *et al*., 1998). The role of hospital environment in the distribution of nosocomial pathogen cannot be overemphasized.

The widespread use of antimicrobials, especially over or inappropriate use of antibiotics, has contributed to an increased incidence of antimicrobial-resistant organisms. Hospital-acquired infections are often caused by antimicrobial-resistant microorganisms. Resistance to antimicrobial agents is a problem in communities as well as health care facilities, but in hospitals, transmission of bacteria is amplified because of the highly susceptible population. The multiple antibiotic resistance (MAR) index confirmed *B. subtilis, B. cereus* and *S. aureus* to be resistant to seven antibiotics. Factors that could be associated with transmission of resistant strains of these microorganisms include poor attention to hygiene, overcrowding, lack of an effective infection control program, and shortage of trained infection control providers (Matar *et al*., 2005).

Conclusion

The result of this study indicated that equipment in x-ray rooms are contaminated by microorganisms. This suggests that contaminated environmental surfaces are reservoirs of these pathogens. *Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis* were multidrug resistant. However, based on this finding, it is clear that the risks of contracting a nosocomial infection may likely occur. Nosocomial infection has been a plague that torments the hospital community, prolonging the number of days patients are hospitalized and often complicates the patient's treatment. The control of microorganisms is therefore of prime importance in hospital and industrial environments.

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