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Isolation and Identification of Microorganisms Associated With Surgical Equipment Used Within the Theatre of Daughters' of Mary Mercy Hospital Ahiaeke in Umuahia, Abia State

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ABSTRACT
Bacterial and fungi species were isolated from cultures made by inoculating corresponding surgical equipment swabs on selective and general purpose media. The media used for the isolation were nutrient agar, Mac.Conkey agar, blood agar, CLED agar, Manitol-salt agar and Sabouraud dextrose agar using streak plate method of isolation. Examples of the surgical equipment checked for microbial contamination were Kidney bowls, forceps, scissors, scrubs, surgical blades, knives, hand washing sinks, retractors, dissecting scissors, needles, blades, syringes etc. The percentage frequency of the bacterial isolate on gallipot was 60%, toothed dissecting forceps 100%, tracing forceps, 0%, needle holder 80%, Chitle forceps 60%, Alice forceps 0%, retractors 80%, corker 0%, curved scissors 80%, kidney dish 60%,cuscus 60%,towel clip 0%, Artery forceps 0%,sponge holding forceps 0%,stitch scissors 0%, non-toothed dissecting forceps 80%. The organisms present on most of the surfaces of the surgical equipment analysed were *Staphylococcus aureus, Klebsiella pneumoniae* and *Candida albicans*. The organisms were confirmed by their affirmative corresponding results to germ tube positive test for *Candida albicans,* catalase positive test for *Staphylococcus aureus* and Voges-Proskaeur plus citrate utilization test positive for *Klebsiella pneumoniae.* Finally, if surgical equipment are not adequately sterilized before utilization during surgeries, it would lead to surgical site infections and every other disease caused by the isolated microorganisms.
Keyword: Isolation, Microorganism, Association, Surgical Equipments, theatre

INTRODUCTION

Microorganisms are found almost on all fomite and most biological surfaces, in extreme environments (Prescott *et al.,* 2013). Amongst the readily colonized fomite surface is that of surgical equipment. Microbial colonization of fomite surfaces can be by commensal bacteria, pathogenic bacteria, prions, yeasts, fungi, protozoans and viruses (Braun, 2008).

A surgical theatre is a room in a hospital or any paramedical facility set aside for the carrying out of surgical operations. It is basically where all forms of surgeries are carried out. The equipment found within any theatre is basically a function of the type of operation carried out within the theatre.

Surgical equipment includes every device, machine, apparatus and material found in the operating theatre and used in performing surgeries. These surgical devices are supposed to be sterile due to the fact that they are used on internal mucosal epithelia and as such can serve as agents of infections and re-infections of these body mucosa. Methods used for the sterilization of these equipment includes boiling, use of detergents, use of disinfectants and antiseptics, use of sterilizing gases e.g. ethylene bromide, use of steam (autoclave) use of chemicals and halogens etc. (De Groote *et al.,* 2016). The presence of these microorganisms on the surface of these fomites could lead to a vast range of complications and infections after the surgeries. These group of infections and diseases arising from

the utilization of these contaminated equipment are referred to as Surgically Acquired Infections/surgical site infection.

Surgically acquired infections/surgical site infection have been a major bane in clinical researches (Hiroshi *et al.,* 2013).

Microorganisms like methicillin-resistant *Staphylococcus aureus, Meningococcus, Penicillum digitatum,* HI virus, Methicillin resistant *Staphylococcus epidermidis,* vancomycin resistant *Enterococci (VRE),* extended spectrum *ß-lactamase (ESBLs)* producing Gram-negative bacteria, *Klebsiella pneumoniae carbapenemase (KPC)* producing Gram-negatives, imipenem resistant *Acinetobacter baumannii,* imipenem resistant *Pseudomonas aeruginosa*, multidrug resistant *Mycobacterium tuberculosis (MDR-TB)* and extremely drug resistant *Mycobacterium tuberculosis (XDR-TB)* are mostly responsible for these surgically acquired infections (Braun, 2008).

Surgically acquired infections/surgical site infection is a subset of Nosocomial infections. Nosocomial infections are also known as hospital acquired infections. These are a group of infections that are gotten during stay in hospital and manifest after discharge from the hospital (Joanne *et al.,* 2013).

Microorganisms found on the surface fomites and other surfaces can be isolated using several isolation techniques. Isolation is the separation of microorganisms on solid or liquid medium aimed at deriving pure culture (Prescott *et al.,* 2016).

Isolation of microorganisms can be done using certain microbiological techniques. These techniques entails Spread plate technique, Pour plate technique, Streaking method, Most probable number etc. The use of selective media, biochemical tests and molecular techniques can be used to further identify and confirm microorganisms.

After microorganisms are isolated, analysis would not be complete until the microorganisms are adequately identified. The techniques used for the identification of isolates is in three arms. This includes: Use of selective media, Biochemical tests and Molecular biological techniques

MATERIALS AND METHODS

Sampling Site

Different surgical equipment in the theatre within Daughters' of Mary Mercy Hospital Ahiaeke in Umuahia, Abia State were obtained. These equipment include kidney bowls, tracing forceps, stitch scissors, retractors, towels Swabs were, needle holders, gallipots, artery forceps, toothed dissecting forceps, non-toothed dissecting forceps, chille forceps, alice forceps, sponge holding forceps, towel clip, cuscus, corker forceps, curved scissors, parker holders etc.

Sample Collection

A sterile swab stick, dipped into normal saline was used to swab the surface of each of the surgical equipment found in the theatre.

Preparation of Working Media

(a) General Purpose Medium

Nutrient Agar

Nutrient agar was prepared following manufacturers instruction (28g in 1000ml of water). The number of working petri plates were calculated and the corresponding volume of medium was prepared. The medium was then poured into the petri dishes and allowed to cool.

(b) Selective Media

Mannitol-Salt Agar

This medium too is also a selective medium. It allows for the growth of *Staphylococcus aureus.* Mannitol-salt agar was also prepared following manufacturers instruction (111g in 1000ml of water). The number of working petri plates were calculated and the corresponding volume of medium was prepared. The medium was then poured into the petri dishes and allowed to cool.

Sabouraud Dextrose Agar

Sabouraud dextrose agar allows for the growth of only fungi species. Sabouraud dextrose agar was prepared following manufacturers instruction (65g in 1000ml of water). The number of working petri plates were calculated and the corresponding volume of medium was prepared. The medium was then poured into the petri dishes and allowed to cool.

Cled Agar and Blood Agar

These media are selective in nature and allows for the growth of only organisms that require too many growth factors. Organisms of this nature are called fastidious microorganisms. The number of

working plates were prepared following manufacturers instruction and poured into already prepared petri plates.

(c) Selective and Differential Medium

Mac.Conkey Agar

This medium is both selective and differential. It selects for the growth of gram negative organisms and gives a different colour from the original colour of the medium. This colour change is due to the presence of certain pH indicators in the medium that detects the presence of acids in the medium. Mac.Conkey agar was prepared following manufacturers instruction (47g in 1000ml of water). The number of working petri plates were calculated and the corresponding volume of medium was prepared. The medium was then poured into the petri dishes and allowed to cool.

Innoculation of Media

The already prepared media contained in petri dishes were inoculated using the swabs form the sample site. The inoculated plates were then incubated at their appropriate temperatures and durations (24 hours incubation period, at 37^0C for bacteria and 7 days incubation period, at 25^0C for the fungi species).

Reading of Petri Plates Results

The already incubated plates, after the completion of incubation duration was observed for the presence or absence of growth.

The total microbial load on the general purpose media and Sabouraud dextrose agar was calculated by counting the number of visible colonies on the petri plates.

Identification of Bacterial Isolates and Fungal Isolates

The isolates from the culture media for bacteria were subjected to gram staining and microscopy and for the fungal isolates, lacto phenol cotton blue stain was used..

Confirmatory Tests for The Bacterial Isolates

Citrate test, Voges-Proskaeur test and Catalase test were used to confirm the bacterial isolates.

(a) Germ Tube Test For Confirming *Candida albicans.*

This test was used to confirm the fungi isolate to be *Candida albicans.*

Candida tropicalis was used as a positive control. A 24 hour broth culture of the fungi isolate was incubated in human blood serum. After a three hour incubation duration, a smear of the broth-serum mixture was observed under the microscope. The presence of germ tubes indicated a positive result while the absence of germ tubes indicated a negative result.

RESULTS

Table 1 shows the presence or absence of growth on MacConkey agar, nutrient agar, blood agar, CLED agar and Manitol-salt agar inoculated with swabs of the different equipment. The percentage frequency of the bacterial isolates was also displayed. The percentage frequency of the bacterial isolate on gallipot was 60%, toothed dissecting forceps 100%, tracing forceps, 0%, needle holder 80%, Chitle forceps 60%, Alice forceps 0%, retractors 80%, corker 0%, curved scissors 80%, kidney dish 60%,cuscus 60%,towel clip 0%, Artery forceps 0%,sponge holding forceps 0%,stitch scissors 0%, non-toothed dissecting forceps 80%,.

The presence or absence of growth on Sabouraud dextrose agar inoculated with swabs of the different equipment and their corresponding frequency of occurrence of fungal isolates as depicted in table 2. Gallipot, non-toothed dissecting forceps, chitle forceps, sponge holding forceps, curved scissors and kidney cuscus showed good fungal colonization as displayed in the fungal growth observed on Sabouraud dextrose agar. Other equipment were free of fungal colonization by both the mycelia and spores.

Table 3 shows the morphology and gram reaction of the bacterial isolates from the different surgical equipment. Gallipot, Toothed dissecting forceps, Chitle and kidney dishes were colonized by gram positive cocci bacteria, which is probably *Staphylococcus aureus.* Non-toothed forceps, retractors, curved scissors, needle holding forceps and cuscus were colonized by gram negative rods, which is probably *Klebsiella pneumoniae.*

The result of the biochemical tests run on the bacterial isolates from the surgical equipment swabs as represented in table 4. Gallipot, Chitle forceps, Toothed dissecting forceps and kidney dishes were colonized by catalase positive Voges-Proskaeur positive and citrate negative bacteria which is probably *Staphylococcus aureus.* Non-toothed forceps, retractors, curved scissors, needle holding

forceps and cuscus were colonized by catalase positive Voges-Proskaeur positive and citrate positive bacteria which is probably *Klebsiella pneumoniae.*

Table 1: Shows the Presence or Absence of Growth on Nutrient agar, MacConkey agar, CLED agar, Blood agar, Mannitol-salt agar and the Frequency of Occurance of Bacterial Isolates.

INSTRUMENT	NA	MA	CLED	BLOOD	MSA	Frequency Of Occurrence Bacterial Isolate.
Gallipot	G	N	N	G	G	60%
Tracing forceps	N	N	N	N	N	0%
Toothed dissecting forceps	G	G	G	G	G	100%
Non-toothed forceps	G	G	G	G	N	80%
Needle holding forceps	G	G	G	G	N	80%
Stitch scissors	N	N	N	N	N	0%
Chitle forceps	G	N	N	G	G	60%
Alice forceps	N	N	N	N	N	0%
Retractor	G	G	G	G	N	80%
Sponge holding forceps	N	N	N	N	N	0%
Towel clips	N	N	N	N	N	0%
Corker forceps	N	N	N	N	N	0%
Curved scissors	G	G	G	G	N	80%
Artery forceps	N	N	N	N	N	0%
Kidney dish	G	N	N	G	G	60%
cuscus	G	G	N	G	N	60%

Keys: G: Growth. BA: Blood Agar N: No Growth. MSA: Mannitol-Salt Agar MA: Mac.Conkey Agar NA: Nutrient Agar

The Presence or Absence of Growth on Sabouraud Dextrose Agar with their Corresponding Possible Organism as depicted in table 2.

INSTRUMENT	SDA	GERM TUBE	POSSIBLE FUNGI
Gallipot	growth	formed	*C. albicans*
Tracing forceps	no growth	not formed	nil
Toothed dissecting forceps	no growth	not formed	nil
Non-toothed dissecting forceps	growth	formed	*C. albicans*
Needle holding forceps	no growth	not formed	nil
Stitch scissors	no growth	not formed	nil
Chitle forceps	growth	formed	*C. albicans*
Alice forceps	no growth	not formed	nil
Retractor	no growth	not formed	nil
Sponge holding forceps	growth	formed	*C. albicans*
Towel clips	no growth	not formed	nil
Corker forceps	no growth	not formed	nil
Curved scissors	growth	formed	*C. albicans*
Artery forceps	no growth	not formed	nil
Kidney dish	no growth	not formed	nil
cuscus	growth	formed	*C. albicans*

KEYS

SDA: Sabouraud Dextrose Agar

Table 3: Morphological and Biochemical test of the Bacterial Isolates from the Different Surgical Equipment.

INSTRUMENT	GRAM REACTION	CATALASE	VOGES PROSKAEUR	CITRATE	PROBABLE ORGANISM
Gallipot	positive cocci	Positive	Positive	Negative	*Staphylococcus aureus*
Toothed dissecting forceps	positive cocci	Positive	Positive	Negative	*Staphylococcus aureus*
	negative rod	Positive	Positive	Positive	*Klebsiella pneumoniae*
Non-toothed dissecting forceps	negative rod	Positive	Positive	Positive	*Klebsiella pneumoniae*
Chitle forceps	positive cocci	Positive	Positive	Negative	*Staphylococcus aureus*
Retractor	negative rod	Positive	Positive	Positive	*Klebsiella pneumoniae*
Curved scissors	negative rod	Positive	Positive	Positive	*Klebsiella pneumoniae*
Kidney dish	positive cocci	Positive	Positive	Negative	*Staphylococcus aureus*
cuscus	negative rod	Positive	Positive	Positive	*Klebsiella pneumoniae*
Needle holding forceps	negative rod	Positive	Positive	Positive	*Klebsiella pneumoniae*

DISCUSSION

Bacterial and fungal isolates were obtained from surgical equipment used in the theatre of Daughters' of Mary, Mother of Mercy Hospital Ahiaeke Ndume in Abia State.

The essence of cleaning surgical equipment is basically to eliminate these pathogenic microorganisms from the surfaces of these equipment, since their presence on these equipment may lead to surgical site infections. In a similar work done by Saito, (2013), it reported that the presence of microorganisms on the surfaces of already sterilized surgery equipment may lead to surgical site contamination and infection. The observed strong microbial contamination of the surgical equipment as observed from the findings of this work, may be due to the ability of microorganisms to gradually recover in surgical field and cause microbial contamination of sterilized surgical equipment (Saito, 2013). In previous research works done, it was reported that most surgical equipment contamination occurred during the surgical procedures proper through the normal flora of the patient, the environment of the theatre and also, the surgical gloves worn by surgeons during surgeries. These routes may play a role as fomites transmission means for these contaminating microbes (Saito, 2013). This also may be the reason while several bacterial and fungal isolates were isolated from these surgical equipment.

CONCLUSION

The presence of microorganisms on the surface of surgical equipment after sterilization is a major problem in clinical medicine. This is because these organisms present on the surface of these equipment become introduced into deep body tissues. These organisms readily cause infections and several complications seen after surgeries. They are responsible for hospital acquired/ surgically acquired/ surgical site infections. Therefore adequate sterilization of surgical equipment should be ensured to avoid this fast spreading menace.

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