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ORIGINAL ARTICLE

A comprehensive study on infection control and liquid waste management in mortuaries

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Abstract

The present study was carried out to assess the biohazard status of waste generated in mortuary complex of a tertiary care hospital. Different methods were employed for isolating microbiota from surface, air, and sewers of mortuary complex. Antimicrobial resistance patterns of all isolated organisms were studied. Disinfection trials were carried out with different concentrations of household bleach, sodium hypochlorite and formaldehyde for liquid waste, surfaces, and air quality, respectively. It was established that 3.33% sodium hypochlorite was most effective for surface sterilization; formaldehyde fumigation once in week was found most satisfactory for maintaining air quality and equal quantities of 10% household bleach was found economically feasible for pre-treatment of hazardous liquid waste generated in mortuaries.

Keywords

Bio medical waste management; Antibiotic resistance; Microbiological surveillance; Safe mortuary practices.

Introduction

Mortuaries are one of the important hotspots in generating high grade infectious biomedical waste. It is very pertinent that large quantities of solid and liquid hazardous waste are generated in a mortuary setting during the process of handling, depositing, and dissecting of dead bodies as part of professional work. Improper handling and managing such waste is a threat for health care workers, community and environment at large. It is very much necessary to quantify the risk in first place and to come up with economically feasible solutions to reduce the risk of any ill effects to all of us working in mortuaries.

Materials and Methods

Place of the study: ACSR Government Medical College and DSR Govt. General Hospital is a tertiary care institution in Nellore city catering to an average of thousand autopsies in a calendar year. Mortuary complex is situated in a separate building within the premises of the institution which includes main autopsy theatre with 4 autopsy tables, cold storage room, decomposed bodies room, central reception facility, inquest room, viscera room, histopathological examination E room, morticians and doctor's rooms with washrooms. Surface disinfection is routinely carried out with commonly available detergents. However, hospital grade phenolic compounds or calcium hypochlorite are occasionally used for that purpose. The study was conducted to perform a qualitative analysis of microbiological contamination in mortuary premises and liquid waste generated from mortuary and to study antimicrobial resistance patterns of bacterial cultured from samples collected from surface, air and sewers in mortuary.

For surface contamination: Ten surfaces were identified based on the frequency of personnel touching those surfaces. Multiple samples for each surface were collected for identifying aerobic bacteria and anaerobic bacteria using sterile swabs and under strict aseptic precautions. Swabs were transported to the central microbiology facility and inoculated within half an hour. Blood agar, MacConkey agar and nutrient agar were used for inoculating samples for aerobic bacteria and Robertson cooked meat broth media is used for inoculating samples for anaerobic bacteria. The plates inoculated for growth of aerobic bacteria were incubated in aerobic environment at 37 degrees centigrade in BOD incubator for 24 hours. Candle jar method was used to create anaerobic environment for growth of anaerobic bacteria at room temperature and the total incubation period was 5 days; followed by identification of species of bacteria grown and their antimicrobial resistance patterns were studied using Kirby Bauer Disc Diffusion method. The Muller Hinton Agar plates used were incubated at 37 degrees centigrade for 16-18 hours in BOD incubator. The standard used for the test and interpretation of results is based on 2019 CLSI Antimicrobial susceptibility testing. The zones of complete growth inhibition were measured by ruler. The results were reported as Susceptible(S), Intermediate (I) and Resistant(R).

The swabs were collected from the following sites - autopsy table 1, autopsy table 2, stretcher 1, stretcher 2, table, bin,

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instruments, tap, door handle inside, door handle outside, floor of decomposed bodies room, dead body freezer inside, dead body freezer outside and floor of autopsy theatre. All the swabs which were collected, were packed, labelled, and immediately sent to microbiology laboratory under strict aseptic precautions. After carrying our disinfection with serially increasing concentrations of freshly prepared lab grade sodium hypochlorite. Swabs were repeated to finally arrive at no growth detected on culture and thereby standards were prescribed. Four different concentrations of sodium hypochlorite 0.025%, 0.05%, 0.5% and 3.33% were used for this purpose. Each specific concentration was used for 15 days and repeat swabs were collected at the end of every two weeks. The simple formula of $C_1V_1 = C_2V_2$ is used for preparing different concentrations of sodium hypochlorite i.e., concentration multiplied by volume of one solution being equated with other.

For air contamination: Settle plate method was employed for assessing the air quality inside the autopsy theatre. A total of 8 blood agar petri dishes were used for this purpose, four test plates and four control plates were placed at the four corners of the room as per 1/1/1 scheme for one hour. After qualitative analysis of species of bacteria grown upon culture, their antimicrobial resistance patterns were studied. Disinfection of the autopsy theatre was carried out by fumigation with formaldehyde. Formaldehyde gas is generated by adding 150 g of kmno4 to 280 ml formalin for every thousand cubic feet of room volume. Room was sealed for 48 hours and then complete aeration was done and fumigant vapour was nullified by ammonia vapour. Temporary arrangements were made for conducting autopsies in the adjoining room during the fumigation protocol. Fumigation was carried out every fortnight for one month and the settle plate method was employed for assessing air quality. Later, fumigation protocol was carried out once in a week for two weeks and settle plate method was employed once again to assess the air quality. Based on the results of the fumigation protocols, standards were prescribed.

For Liquid waste generated from mortuary: Five samples of water 5ml each were collected from sewer lines of mortuary at 100m, 200m, 300m, 400m and 500m from the autopsy tables. Qualitative analysis of species of bacteria grown upon and their antimicrobial resistance patterns were studied. All water samples collected were prior to sewage treatment only. Methods routinely used for bacteriology of water were employed for qualitative and quantitative reporting. Presumptive coliform count method (Multiple tube method) using purple MacConkey broth was employed. Disinfection with serially increasing concentrations of common bleach was engaged in laboratory setting to see for maximum disinfection and standards were prescribed.

Results

This study was aimed to detect aerobic and anaerobic pathogenic bacteria by way of gram staining and culture and thereby also study the resistance pattern of those organisms. Fungal growth and aerobic spore forming bacilli were not further studied as it was not planned as part of the study. All the swabs received in the microbiology laboratory were processed without any delay. Before culture direct gram stain, pink coloured rod-shaped organisms in single and violet-coloured spherical shaped organisms in pairs and groups were observed abundantly. Occasionally, purple organisms with drum stick appearance and rarely gram-positive bacilli were also observed. Swabs are inoculated on to culture plates (Blood agar and Robertson cooked meat broth medium) and incubated for 24 hours at 37°C -38°C.

All plates including settle plates and surface swab inoculated plates showed polymicrobial growth after 24 hours. Clostridial growth was identified in Robertson cooked meat broth media after 5 days. Within the media, meat turned in to black and emanated foul odour. This was inoculated in to blood agar and incubated at room temperature for 24 hours under anaerobic conditions using candle jar method.

Table 1: Profile of microorganisms grown at various sites in mortuary

Site	Organisms grown
Autopsy table 1	EF, EC, PA, ES, CS, KP, SA, AB
Autopsy table 2	EF, EC, PA, ES, KP, SA, AB
Stretcher 1	EF, EC, CS, KP, SA, AB
Stretcher 2	EF, EC, PA, ES, CS, KP, SA, AB
Bin	EF, EC, PA, AB
Instruments	EF, EC, PA, ES, CS
Tap	EF, EC, PA, ES, CS, KP, SA, AB
Door handle inside	EF, EC, PA, ES, CS, KP, SA, AB
Door handle outside	EF, EC, PA, ES
Settle plate 1	KP, SA, AB
Settle plate 2	SA, AB
Settle plate 3	KP, SA, AB
Settle plate 4	KP, SA, AB
Floor of room for decomposed bodies	EF, EC, PA, ES, KP, SA, AB
Dead body freezer inside	PA, ES, CS, KP, SA, AB
Dead body freezer outside	EC, PA, ES, KP, SA, AB
Floor of autopsy theatre	EF, EC, PA, ES, CS, KP, SA, AB
Settle plate controls (1-4)	Nil. These were part of ensuring quality control of media.

Enterococcus faecium, (EF); Staphylococcus aureus, (SA); Klebsiella pneumoniae, (KP); Acinetobacter baumannii, (AB); Pseudomonas aeruginosa, (PA); Enterobacter species (ES); Clostridia Species (CS); E Coli (EC)

Characteristic swarming growth appearance was seen and the organisms were presumptively confirmed as clostridia with culture gram's stain.

Table 2: Profile of microorganisms grown from samples collected from sewer.

Water collected from Sewer with respect to distance from autopsy table	Organisms isolated
100m	EF, KP, SA, EC
200m	CS, EC, PA, AB
300m	EC, KP, EF
400m	EC, KP, EF
500m	EC, KP

Enterococcus faecium, (EF); Staphylococcus aureus, (SA); Klebsiella pneumoniae, (KP); Acinetobacter baumannii, (AB); Pseudomonas aeruginosa, (PA); Enterobacter species (ES); Clostridia Species (CS); E Coli (EC)

Table 3: Microbial growths after using different concentrations of sodium hypochlorite as surface disinfectant

Site	0.025%	0.05%	0.5%	3.33%
Autopsy table 1	AG	AG	MG	NG
Autopsy table 2	AG	MG	MG	NG
Stretcher 1	AG	MG	MG	NG
Stretcher 2	AG	MG	NG	NG
Bin	AG	MG	MG	NG
Instruments	AG	MG	MG	NG
Tap	AG	AG	MG	NG
Door handle inside	AG	MG	MG	NG
Door handle outside	AG	MG	MG	NG
Floor of room for decomposed bodies	AG	NG	NG	NG
Dead body freezer inside	AG	MG	MG	NG
Dead body freezer outside	AG	NG	NG	NG
Floor of autopsy theatre	AG	MG	MG	NG

AG- Abundant Growth (> or = 5 CFU/cm2); MG- Minimal Growth (1- <4 CFU/cm2) ; NG- Nil Growth.

Table 4: Microbial Growth in settle plates on third day after fumigation with formaldehyde

Site	Fumigation once in 15 days	Fumigation once in 7 days
Settle plate 1	AG	MG
Settle plate 2	MG	MG
Settle plate 3	AG	MG
Settle plate 4	MG	MG

AG- Abundant Growth (> or = 5 CFU/cm2/ hr); MG- Minimal Growth (1-<4 CFU/cm2/ hr); NG- Nil Growth.

Table 5: Disinfection of liquid waste from mortuaries with different percentages of household bleach

Water collected from Sewer with respect to distance from autopsy table	5%	7.5%	10%
100m	AG	MG	MG
200m	MG	MG	NG
300m	NG	MG	NG
400m	AG	MG	NG
500m	MG	MG	NG

AG- Abundant Growth; MG- Minimal Growth; NG- Nil Growth. (Enumeration criteria in accordance with presumptive coliform method)

Table 6: Antibiogram showing resistance patterns of organisms isolated from different sites

Antibiotic	*Enterobacter species (ES)*	*Pseudomonas aeruginosa, (PA)*	*Acinetobacter baumannii,* (AB)	*Klebsiella pneumoniae,* (KP)	*Staphylococcus aureus,* (SA)	*Enterococcus faecium ,(EF)*	*E Coli (EC)*
Amoxycyllin/ Clauvlanate(30 µg)	S	R	R	R	S	R	R
Amikacin(30 µg)	R	S	R	S	R	S	R
Cotrimoxazole(25 µg)	R	R	R	R	S	R	R
Cefotaxime(30 µg)	R	S	R	R	R	S	S
Imipenam(10 µg)	R	R	R	R	R	S	R
Levofloxacin(5 µg)	R	S	R	R	R	S	R
Piperacillin / Tazobactum(10 µg)	R	S	S	R	R	R	R
Carbencillin(100 µg)	R	R	R	R	R	R	R
Oxacillin(1µg)	-	-	-	-	R	R	-
Colistin(25 µg)	R	S	R	R	R	S	R

R-Resistant; S-Susceptible

Sub culturing was done in MacConkey agar, Nutrient agar, and blood agar. Based on colony characters, a variety of organisms were observed. Subsequently, biochemical reactions were performed to confirm species of microbiota. The data is summarized in Table 1 to Table 3. The following biochemical tests were carried out viz. Catalase Test and Oxidase Test, motility confirmation by hanging drop method, Indole test, Methyl Red Test, Voges-Proskauer *Test* Citrate Test, Urease Test, Triple Sugar Iron Test, (for gram negative organisms). Coagulase Test was used for gram positive organisms (GPC) to differentiate between CoNS (coagulase negative staphylococci) from other GPC. Both slide and tube method were used. Aerobic spores and CoNS grown were not further processed as they are non-pathogenic. CoNS, micrococci, diptheroids and aerobic spore forming bacteria were also identified. However, they were not tabulated because they are not that much

clinically significant. Due to unavailability of cefoxitin at the time of study, oxacillin was used to differentiate between MSSA and MRSA. The organisms were resistant to oxacillin and several other drugs but they were not included in the table to reduce the unnecessary volume of information. Colistin was selected at the end only as an experimental trial after several drugs tested to be resistant.

After fumigating with formaldehyde, settle plate method was used on the third day to assess disinfection. Fumigation was performed fortnightly initially and once in a week later and results were compared (Table 4). Liquid waste from the sewer was treated with equal quantities of 5% household bleach, 7.5% household bleach and 10% household bleach and was allowed to settle for half an hour and cultures were done from that solution to assess disinfection capabilities of bleach of different concentrations (Table 5 & 6).

Discussion

A thorough search of available literature for microbiological surveillance of autopsy theatres and liquid waste management in mortuaries revealed very few results. However, methods employed for routine operation theatre sterilization can be extrapolated for the purpose of prescribing guidelines for autopsy theatres. After analysing the results of the study, it is not an exaggeration to state that unless a proper infection control plan is worked out for mortuaries, they will become hot spots for emerging superbugs. Because of availability or rich nourishment for bacteria hospital sewers containing untreated liquid hazardous wastes may become sites for resistance transfer between species.

E Coli and Klebsiella demonstrated high degree of multidrug resistance in untreated hospital sewage in a study by Kabir.[1] Our study also demonstrated multi drug resistance in Klebsiella and E Coli isolated from sewers. Settle plates from autopsy theatres showed growth of Staph aureus in our study and the same organisms were identified by a study conducted by Javed et al.[2] *Enterococcus faecium*, (EF) *Staphylococcus aureus*, (SA) *Klebsiella pneumoniae*, (KP) *Acinetobacter baumannii*, (AB) *Pseudomonas aeruginosa*, (PA) *Enterobacter* species (ES) *Clostridia Species (CS) E Coli (EC)* were detected from cultures of sewer lines in our study which is similar to Numberger D et al.[3] Unfortunately, autopsy theatres are not considered on par with surgical OTs. However, some sort of minimum standards of infection control are to be followed for mortuary complexes as well.

Conclusion

In the light of glaring evidence of high risk for everyone who work in mortuaries, it is high time we take a pledge for clean and safe mortuary practices. Bio medical waste management protocols should be drawn up for all mortuary complexes along with a contingency plan for infection control. Unless the work environment is made more ergonomic and safer it would be exceedingly difficult to find doctors, morticians and ancillary staff ready to work in mortuaries in near future.

Recommendations[4-13]

The following Ten Commandments are to be followed for welfare of all of us.

1. All surfaces inside mortuary complex should be mopped with 3.33% sodium hypochlorite solution at the end of daily work and if possible, between cases as well.
2. All major rooms in mortuary complex are to be fumigated with at least formaldehyde once a week. Since formaldehyde is being held as a carcinogen it is advisable to search for a cheap yet effective fumigant.
3. Liquid waste generated from mortuary is to be ideally pre-treated in an effluent treatment facility before release from the hospital premises. If such facilities are not available a pre-treatment with 10% household bleach before releasing into municipal sewage is to be followed during the transition period.
4. All personnel in mortuary are to be trained in infection control practices. A proper disinfection action plan includes assessment, cleaning, washing, disinfection, and evaluation. A customized plan may be developed for each setting or the general guidelines in this document may be followed.
5. All mortuary personnel should be vaccinated for vaccine preventable diseases with great caution.
6. Every autopsy complex should have three or more dissection theatres separately earmarked for decomposed cases, infective cases, and routine cases etc.
7. The design of autopsy complex shall have arrangements for air conditioning and proper exhausts are to be fitted preferably close to the ground.
8. All care should be taken to ensure that solid biomedical waste like linen, casts, slabs, catheters etc. are properly segregated at source into respective bins.
9. A septic tank like arrangement is better for allowing the liquid waste generated in mortuaries which can be connected to a pre-treatment facility before letting off the effluents into municipal sewage.
10. It is always advisable to have a team of doctors including microbiologist, hospital administrator and autopsy surgeon for proper mortuary management with respect to infection control.

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