

Original Research Article

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## Contaminated Outer Surfaces of Clinical Sample Containers Received at a Public Tertiary Healthcare Centre Microbiology Laboratory: Need for Re-Emphasis on Occupational Safety in the Developing World

T.M. Nandan<sup>1\*</sup>, R. Ravikumar<sup>2</sup>, G. Latha<sup>1</sup>, S. Nagarathna<sup>2</sup>,  
H.B. Veenakumari<sup>2</sup> and K. Vidyasagar<sup>3</sup>

<sup>1</sup>Department of Microbiology, PES Institute of Medical Sciences and Research, Kuppam, India

<sup>2</sup>Department of Neuromicrobiology, National Institute of Mental Health and Neuro Sciences, Bengaluru, India

<sup>3</sup>Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, Bellur, India

\*Corresponding author

### A B S T R A C T

#### Keywords

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Although clinical sample containers can be perceived to be harbouring pathogens on their outer surfaces, a vast majority of healthcare personnel appear to be accustomed to keeping this fact out of their minds due to unknown reasons. The current study provides a cross sectional view of this complex, often ignored, scenario of overlooking subtle infection control practices that may lead to acquisition of potentially infectious bacteria. The study was conducted using 51 clinical sample containers received at a public tertiary healthcare centre Microbiology laboratory between February and April 2013. Samples were collected from the outer surfaces of the containers and were immediately inoculated and subcultured on to necessary plating media, the organisms isolated, and their antibiotic susceptibility patterns elucidated. Virtually all the clinical sample containers yielded one or the other organisms from their outer surfaces. Majority of the Gram positive isolates were Methicillin Resistant Coagulase Negative Staphylococci and of the Gram negative isolates were coliforms with over half of the isolates being multiply antibiotic resistant. The present study tries to provide the scientific healthcare community and the community at large, with the much needed re-emphasis about the routinely neglected aspect of occupational risk to healthcare personnel.

### Introduction

Although clinical sample containers can be perceived to be harboring pathogens on their surfaces it is a common sight that a vast majority of healthcare personnel including the general populace of the community, appear to be accustomed to keeping this fact out of their minds due to unknown reasons. Within the complex struggle against time and limited resources to achieve uniform healthcare to all

in the healthcare services framework, it is often inevitable that the sample collection, labeling, filling the lab requests, etc., take place under minimal direct expert supervision, in many local settings. Many a time, it may happen even because of lack of awareness and minimal adherence to basic, general guidelines on hospital infection control practices among hospital staff and

patients of varied socio-economic and educational background. As various clinical sample containers may be handled by more than one individual from collection through transit to respective laboratory sections, it is imperative to demonstrate that outer surfaces of containers become contaminated with the sample strains and/or the healthcare personnel's or hospital environmental, transient/otherwise flora, including those potentially infectious, often lately, the drug resistant ones, especially when they are not individually wrapped (Hota, 2004; Mbithi *et al.*, 1992; Maule, 2000; Ansari *et al.*, 1988; Noskin *et al.*, 1995).

The current study provides a cross sectional view of this complex, often ignored, scenario of overlooking subtle (because microscopic) infection control practices that may lead to a slow but sure, dreaded human acquisition of routinely cultivable potentially infectious bacteria, let alone other difficult to culture pathogenic agents like mycobacteria (Allen *et al.*, 1983), chlamydiae (Novak *et al.*, 1995), viruses (Gordon *et al.*, 1993; Sattar *et al.*, 1987; Mahl *et al.*, 1975; Sattar *et al.*, 1986; Gordon *et al.*, 1993; Bean *et al.*, 1982; Gwaltney *et al.*, 1982), rickettsiae (Pike, 1979) fungi, parasites (Pike *et al.*, 1965) and prions (Mari DeMarco, 2015), etc., and the consequences thereby, within and often, without any healthcare facility. It also therefore attempts to throw insight into the most needed promotional health-education demand regarding the pathogenic microbial epidemiology in healthcare settings, laboratory or otherwise, especially in developing and under-developed economies.

## Materials and Methods

The study has been an observational prospective cross-sectional one which estimates the prevalence of cultivable aerobic bacterial pathogens on the outer surfaces of

clinical sample containers. The study was conducted sampling in random 51 clinical sample containers received at an urban public tertiary healthcare centre microbiology laboratory during a three month period between February and April 2013. Sample size was arrived at for an estimated prevalence of >95% with 95% confidence level and a precision of 0.05 (Singh *et al.*, 2014; Veena Kumari *et al.*, 2012).

The sample swabs were aseptically collected (Allen *et al.*, 1983) from the outer surfaces of clinical sample containers as soon as they were received at the sample receiving section of the microbiology laboratory with sterile cotton tipped swabs moistened with sterile normal saline. For the urine, tracheal secretions, tube tip and CSF samples: the mid-portion used to hold the container (approximate height, 5 cm with a diameter, 4 cm) by hand, the cap, the neck of the containers to a depth approximately 10mm below the cap across the circumference of the cylindrical containers and for the pus swabs: the longer outer container tube that screw caps the swab stick fixed to the cap (approximate length, 15 cm and diameter 1cm), the cap itself, were swabbed and immediately inoculated into thioglycollate broth (routinely used for inoculation of samples) and incubated at 37°C for 48 hours. They were then subcultured on to suitable plating media like blood agar, nutrient agar and MacConkey's agar. Plain saline soaked sterile swabs were periodically similarly inoculated to double check sterility of the saline and the swab tips.

The isolates were identified using standard microbiologic methods to isolate aerobic and facultative anaerobic organisms including Gram staining, coagulase, oxidase, catalase tests, MMTP reactions, citrate utilization, urease production, etc. Antibiotic susceptibility testing of the isolates was

performed by Kirby Bauer disk diffusion method using appropriate classes of antibiotics for the Gram varieties. Screening antibiotic susceptibility tests like the cefoxitin screen for penicillin resistant *Staphylococci*, etc., were performed. Standard interpretative guidelines including that of CLSI were followed (CLSI, 2013).

## Results and Discussion

Table 1, lists the total number of clinical sample containers along with the number of them harboring organisms, pathogens, of medical cases, of surgical cases, of male ward cases and of female ward cases. Forty nine of 51 swabs (96 %) from the outer surfaces of clinical sample containers yielded one or the other routinely cultivable organism with the remaining only 2 of them being sterile. The number of containers harboring pathogens on their surfaces was 28 out of 51(55 %) with 4 of them yielding two pathogens per container surface. Containers from surgical cases and female ward cases had greater number of pathogens on their outer surfaces. Table 2 enlists the total number, pathogenic and non-pathogenic isolates from the outer surfaces of all the sample containers. Sample containers of medical and surgical cases that harbored pathogens on their surfaces were 53 % and 56 % respectively. Fifty % of the male patients' and 62 % of the female patients' sample containers had pathogens on their surfaces.

Table 3 shows the types of samples received and available for the random sampling process. It also shows the percentage of each such sample containers that grew organisms on their outer surfaces. The number of each type of samples received reflected the usual respective sample load in the laboratory. CSF sample containers grew the least number of organisms on their outer surfaces. In total, 32 pathogenic organisms were isolated with 16 each (50%) belonging to either of the Gram

reactions. Graph 1 and Table 4; show the relative numbers of isolated organisms. The usually non-pathogenic aerobic spore bearers were recovered from majority of the containers. Coagulase negative *Staphylococci* and *Enterobacteriaceae* predominated among the pathogens. Non-fermenters were also isolated from a few of the sample containers. Incidentally, the gram positive pathogens equaled the number of gram negative isolates.

Majority (50 %) of the Gram positive isolates were Methicillin Resistant Coagulase Negative *Staphylococci* and of the Gram negative isolates were coliforms (81 %). All the isolates exhibited varied antibiotic susceptibility patterns. The Gram positives showed the greatest resistance to beta lactams at 50 %, and the least resistance to the aminoglycoside gentamicin at 25 %. Those resistant to  $\geq 3$  classes of antibiotics were 31 %. Graph 2 shows the relative percentages of resistance exhibited by the Gram positive isolates to the commonly used different classes of antibiotics. None of the isolates were vancomycin resistant, though greater number of them showed resistance to beta-lactams (cefoxitin screen), the anti-metabolite co-trimoxazole, the fluoroquinolone ciprofloxacin, the macrolide erythromycin and the aminoglycoside gentamicin in the decreasing order of resistances.

Graph 3 shows the relative percentages of resistance exhibited by the Gram negative isolates to the commonly used different classes of antibiotics. The greatest number of isolates showed resistance to ampicillin, ceftriaxone and ceftazidime, hinting towards the prevalence of ESBLs, slightly lesser resistance to the anti-metabolite co-trimoxazole and the aminoglycoside gentamicin and the least resistance to the fluoroquinolone ciprofloxacin and tetracycline. The Gram negatives had the highest (75 %) and the least (25 %)

resistances to ampicillin, and ciprofloxacin and tetracycline respectively. Those resistant to  $\geq 3$  classes of antibiotics were 56 %.

Table 5 shows the presence of multiply drug

resistant organisms among the isolates. The Gram negative isolates showed greater degree of resistance among both those that were resistant to 2 classes and  $\geq 3$  classes of antibiotics.

**Table.1** Number of organisms isolated

Total no of sample containers(n=51)	Number	Percentage
No of containers harboring organisms on their outer surfaces	49	96%
No of containers harboring pathogens on their outer surfaces	28	55%
No of containers harboring pathogens on their outer surfaces: from medical cases	10/19	53%
No of containers harboring pathogens on their outer surfaces: from surgical cases	18/32	56%
No of containers harboring pathogens on their outer surfaces: of male patients	15/30	50%
No of containers harboring pathogens on their outer surfaces: of female patients	13/21	62%
No of containers harboring pathogens on their outer surfaces same as that in the sample	2/32	6%

**Table.2** Number of pathogenic and non-pathogenic isolates

Number of pathogenic isolates	32	39%
Number of non-pathogenic isolates	51	61%
Total number of isolates	83	100%

**Table.3** Types of sample containers and the number of them showing growth

Type of Sample container	Number	Number showing growth of organisms on outer surfaces	Percentage
Urine	18	12	67%
Tracheal	12	6	50%
CSF	12	4	33%
Tip	4	3	75%
Pus	5	3	60%

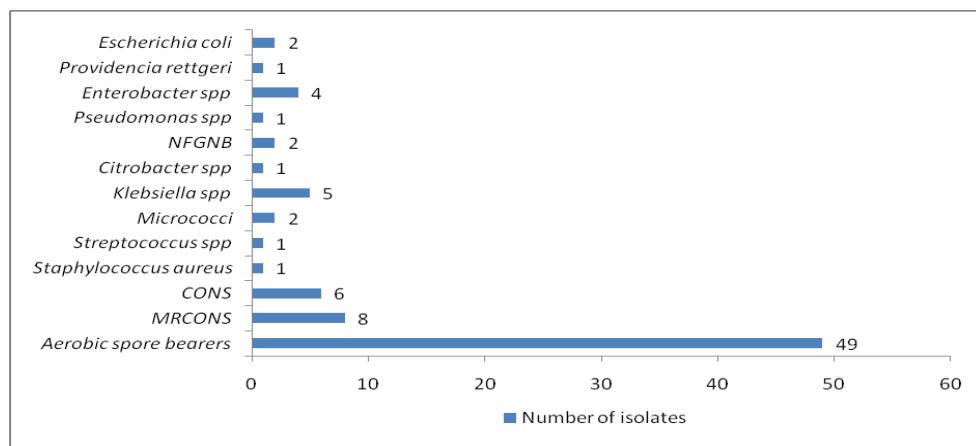
**Table.4** Number and percentages of the Gram positive and negative isolates

Pathogenic Gram positive isolates	Number	Percentage
MRCNS	8	50%
CONS	6	38%
<i>Staphylococcus aureus</i>	1	6%
(β) <i>Streptococcus</i> spp	1	6%
Total	16	100%
Pathogenic Gram negative isolates	Number	Percentage
<i>Klebsiella</i> spp	5	31%
<i>Citrobacter</i> spp	1	6%
NFGNB	2	13%
<i>Pseudomonas</i> spp	1	6%
<i>Enterobacter</i> spp	4	25%
<i>Providencia rettgeri</i>	1	6%
<i>Escherichia coli</i>	2	13%
Total	16	100%

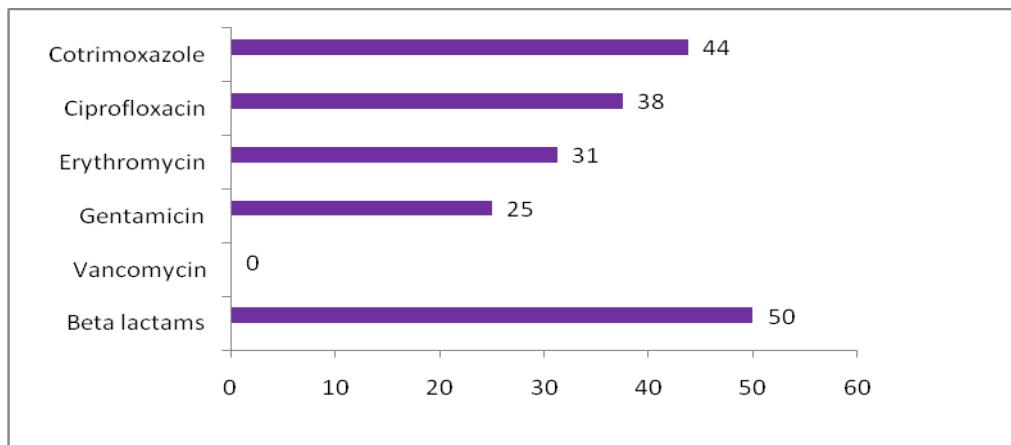
**Table.5** Presence of multiply drug resistant isolates

	Gram positives	Gram negatives
Percentage resistance to 2 classes of antibiotics	44%	63%
Percentage resistance to ≥ 3 classes of antibiotics	31%	56%

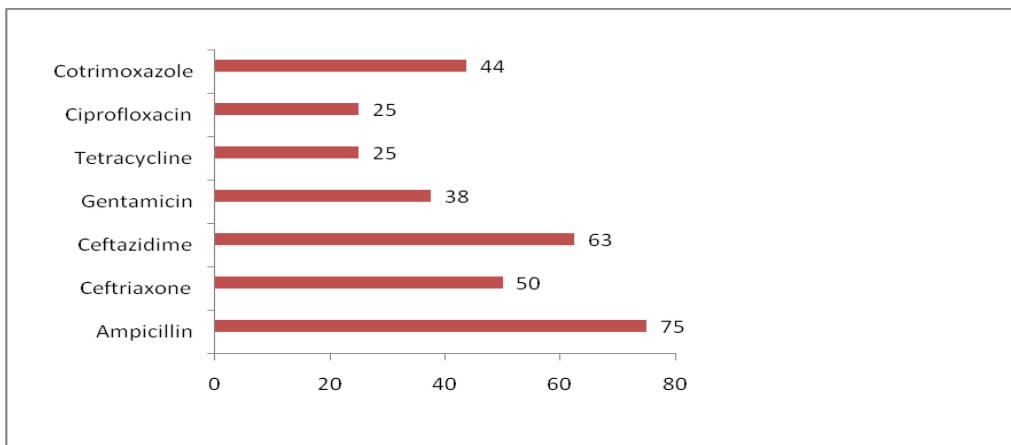
**Graph.1** Relative numbers of all the isolates



**Graph.2 Percentage resistance among Gram positive isolates**



**Graph.3 Percentage resistance among Gram negative isolates**



The present study highlights that the sample containers harbor a wide array of drug resistant pathogens on their outer surfaces that pose risk of transmission to all those who handle them right from the time of collection, transport, receipt at a laboratory (Microbiology in this case) and of course within the laboratory as well.

In the study on sputum sample containers (Allen *et al.*, 1983), 14 % contamination with sputum material was detected on the same sample container outer surfaces. The other aspect of the same study detected 6.5% contamination with *Mycobacterium tuberculosis* among the positive sample

containers. There is also probability of aggregation of microbes while they desiccate on the surfaces of containers posing the threat of becoming droplet nuclei within the laboratory area especially when handled to move or open the lids (Darlow, 1972). Both these findings are remarkable given the increasing number of healthcare associated infections to the present time, both in the developed and developing countries. The finding is more alarming to the latter due to complex cross contamination dynamics of hospital bugs involving the healthcare personnel and patients as well, as picked up by the present study.

A previous study on sputum smears (Allen *et al.*, 1981), tubercle bacilli were cultured from heat-fixed sputum smears and showed that laboratory staff may unknowingly handle dried infectious sputum material without protection e.g., from lack of bio-safety cabinet or of course also the inadequate/non-provision of hand gloves as happen in resource poor settings.

A similar study (Sing *et al.*, 2014) on isolates from various surfaces within a clinical microbiology laboratory attached to a tertiary care medical institution in Mumbai, showed similar proportion of distribution with a predominance of Gram positive organisms like *Bacillus* species, 36 % followed by Coagulase negative *Staphylococcus*, 14 %, *Staphylococcus aureus*, 13 %, *Micrococcus* spp, 9 %, *Pseudomonas aeruginosa* and *Klebsiella* species, 6.5 % each.

A related study done in the same institute (Veena Kumari *et al.*, 2012), on patient case files documented 93% contamination rate with a variety of potentially pathogenic, pathogenic and environmental bacteria in the healthcare setting. Overall, the isolation rates of the potentially pathogenic coagulase negative *Staphylococci* were 45%, the pathogenic: coliforms and non-fermenters were 8 % and 5 % respectively. *Corynebacterium* spp was isolated at 38% as a probable environmental contaminant. Seven of the case files grew two organisms each. The study had also found correlation between the isolates from the case files and the isolates from the clinical samples of the same patients; the present study also documents 6% correlation between the clinical sample isolates and those on the outer surfaces indicating contamination while collection. Contamination was not present in well monitored areas like the neuromedical and neurosurgical intensive care units but was found to be maximum in the difficult to

monitor emergency care and general ward settings. This has been observed in the present study as well by less number of critical samples like CSF, tracheal secretions growing organisms on their outer surfaces (Table 3).

Often ignored, neglected, less publicised, subtle microbial transmission dynamics: Studies on bacteriology of outer surfaces of clinical sample containers and other potential sources of microbial transmission like the healthcare environment of poorly maintained clinical labs, especially the microbiology and virology are sparse from developing countries. Similar conclusive studies implicating exteriors of sample containers as causes of infectious diseases, both healthcare as well as community acquired ones had been in vogue in the past in the now developed world (Singh, 2009). The rich economies appear to have learned from their experiences and have successfully overcome this issue by strict implementation of infection control practices, which are now probably part of their mere routine in the form of stringent though feasible policies and standard operating procedures. This is perceivable by the developing economies now adopting and referencing these international standards related to bio-safety and healthcare delivery in general through various accreditation schemes, both national and international (Coelho *et al.*, 2015; Sing *et al.*, 2014; Veena Kumari *et al.*, 2012). In view of such schemes leading the world towards normalization of healthcare delivery systems, it is requisite to bridge the gap of this technical know-how about bio-safety among all the trailers even at the grass root level. The authors have felt the need for stressing the importance of apparently neglected aspect of bio-safety in developing economies where patient centred care often overrules the occupational health of healthcare personnel, mainly due to monetary, educational and attitudinal issues, which however can be addressed by right amount of

healthcare education and training at appropriate levels.

As stated above, though the importance of bio-safety had been brought to the fore to the vast span of the healthcare audience of the developed world only since the 1950s, 60s, 70s, through the 80s and 90s, it took quite a long time for implementing strict policies related to the provision of healthy atmosphere for the bio-safe practice of healthcare in general. The principal aim of such formal publications of reports of accidents related to pathogens among both the laboratory and hospital personnel has been to alert the policy makers and the public at large to prevent such fatal and near-fatal incidents of the past, when it was just an optional part of the management system to publish such occurrences (Collins, 1988). In contrast, the developing world had neither the first hand information about microbes in general, let alone the pathogens that cause fatal illnesses, nor the colonial governments and their successors were compassionate and bold enough to educate all their subjects about infectious diseases and their agents except for the age old practice of hygiene among a few sympathetic fellow countrymen. No wonder, the concept of occupational bio-safety takes considerable duration of time to be obviously felt as being practiced in day to day laboratory/ hospital lives.

Containment of infections due to contaminated clinical sample containers: In view of increasing number of healthcare associated infections due to multi-drug resistant pathogenic bacteria, it is worthwhile to prevent unintentional/unwanted exposure to dangerous bugs and/or to their drug resistance inducible genetic materials viz., plasmids and transposons leading to an alarming state of multi-drug resistant “pathogen commensalism” and the resulting untoward effects. Prevention of transmission

of infection is possible at the ward/sample collection level by several ways including adequate cleaning and disinfection of environmental surfaces, use of alcohol hand-rubs and hand-washing with soap and water, clear instructions for collection of urine and stool samples to patients. At the laboratory level, laboratory healthcare personnel can prevent transmission to themselves and others in the labs only when barrier protective measures including wearing hand gloves are strictly adhered to whenever samples/sample containers are handled including the novel judicious use of hand-rubs on the gloved hands whenever possible, e.g., after checking and sorting sample containers due for processing, i.e., when they are not visibly soiled (Nandan *et al.*, 2015).

This will also address the recommendations of ‘standard precautions’ that all samples must be considered potentially infectious (CDC, 1988). The use of zip-lock plastic bags for transporting clinical sample containers would further strengthen the infection control system by preventing accidental spills in unwanted locations although does not guarantee pathogen-free outer surfaces. The use of tissue paper folds to uncap/recap fluid sample containers while processing prevents spillage of drops of samples adherent to the undersurfaces of the caps, which usually does happen (Nandan *et al.*, 2015).

Proper safety instructions to the hospital and laboratory healthcare personnel for collection and handling of samples would also greatly benefit the infection control system. The healthcare management systems must cater to this subtle yet perpetual need by encouraging and providing infrastructure, education and training regarding appropriate use of personal protective equipment and techniques thereby as exemplified above and definitely in more novel ways (CDC, 2012).

In conclusion the present study tries to provide the scientific healthcare community and the community at large, with the much needed awareness about the routinely neglected aspect of epidemiology of healthcare associated, especially the occupational risk to healthcare personnel including microbiology laboratory workers in any healthcare facility in the developing/underdeveloped world. The authors feel that much more needs to be done to improve the present working conditions to promote occupational health in the healthcare delivery system. This approach would go a long way in strengthening the control of any emergent and life threatening infectious diseases of the future that may add on to the financial burden of the economy as well. Optimal awareness about microbial causes and epidemiology of infectious diseases in day to day practice would further reduce the rampant high risk behavior among the members of the community (including healthcare personnel) and would result in their improved living standards on par with the highest possible quality level. Collins, 1988, rightly concluded in his review on "Safety in Microbiology Laboratory" that "only those who are unaware of the facts, and are not themselves at risk, will dismiss these hazards as acceptable or non-existent".

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