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Hospital Wastewater Sludge: An Unaddressed Environmental Reservoir for Emerging and Rare Nosocomial Pathogens

Lodo de aguas servidas de hospital: un reservorio ambiental no planteado para patógenos nosocomiales emergentes y raros

Pallavi Bhatt[1*], Nupur Mathur[1], Anuradha Singh[1], Priyanka Sarkar[2], Pradeep Bhatnagar[3]

1- Environmental, Molecular, Microbiology (EMM) Laboratory, Department of Zoology, University of Rajasthan, Jaipur 302004, Rajasthan, India. Email: pallavi29@gmail.com , nupurmathur123@rediffmail.com, anuradhasingh1994@gmail.com

2- Wellcome/DBT (Indian Alliance) Lab, Asian Healthcare Foundation, Asian Institute of Gastroenterology, Hyderabad 500032, India. Email-priyanka.sarkar14@gmail.com

3- Dean, Faculty of Science, IIS University, Jaipur, Rajasthan, India. Email: Pradeep.bhatnagar@iisuniv.ac.in

*Corresponding author: Pallavi Bhatt, Environmental, Molecular, Microbiology (EMM) Laboratory, Department of Zoology, University of Rajasthan, Jaipur 302004, Rajasthan, India. Email-id: pallavi29@gmail.com, Telephone +91-9828606829

ABSTRACT

Nosocomial infections cause significant mortality and financial losses each year. Most of these infections are caused by multidrug resistant (MDR) opportunistic pathogens and therefore are difficult to treat by standard therapies. Though hospitals are considered as ecological niches for nosocomial pathogens, environmental reservoirs for the same are still underexplored. The present study addressed this issue by systematically profiling the pathogenic diversity of hospital wastewater sludge hypothesized as an important reservoir for nosocomial pathogens within a hospital setting using Illumina Miseq Next Generation Sequencing (NGS) approach. The NGS data showed that i) nosocomial pathogens dominated the hospital sludge bacterial profile and majority of them fell in the category of either emerging or rare pathogens ii) Majority of the pathogens formed part of the low abundant microbiota represented by 3.56% of the reads iii) Nearly 14% of the reads were represented by the unculturable bacteria iv) Of the 580 species-level operational taxonomic units (OTUs) identified in this study, 166 matched with potential human pathogens v) *Enterobacter cloacae* (56.45%) was the most dominant species followed by *Pseudomonas putida* (6.07%), *Fusobacterium ulcerans* (3.08%) *Acidaminococcus fermentans* (2.03%) respectively. *Aeromonas hydrophila*, *Klebsiella pneumoniae* and *Pantoea agglomerans* formed the less dominant species. This study points towards the catastrophic effect on public health and environment that may result from the co-treatment of hospital wastewater with domestic wastewater in municipal wastewater treatment plants and the use of resultant sludge in agriculture which is a common method of sludge disposal practiced in developing countries.

Keywords: Hospital sludge, Next Generation Sequencing, Nosocomial Pathogens, Emerging Pathogens, Rare Pathogens.

RESUMEN

Cada año, las infecciones nosocomiales causan pérdidas financieras y de mortalidad significativas. La mayoría de estas infecciones se causan por patógenos oportunistas multirresistentes (MDR, del inglés multidrug-resistant), lo que hace que sean muy difíciles de tratar con tratamientos convencionales. Aunque se considera a los hospitales como nichos ecológicos para patógenos nosocomiales, aun los reservorios ambientales están inexplorados para el mismo propósito. El presente estudio plantea este problema mediante la elaboración de un perfil sistemático sobre la diversidad patógena del lodo de aguas servidas de hospital, en la cual se hipotetiza como

un reservorio importante para patógenos nosocomiales dentro un contexto de hospital mediante el empleo del MiSeq de Illumina y con la tecnología de Secuenciación de Nueva Generación (NGS, por sus siglas en inglés). Los datos del NGS revelaron que I) Los patógenos nosocomiales dominaban el perfil bacteriano del lodo de hospital y la mayoría de ellos entraban en la categoría, ya sea de patógenos emergentes o raros; II) La mayoría de los patógenos formaron parte de la microbiota poco abundante representada por el 3,56% de las lecturas de ADN; III) Cerca del 14% de las lecturas de ADN fueron representadas por las bacterias no cultivables; IV) De las 580 unidades taxonómicas operacionales (UTO del inglés Operational Taxonomic Unit) a nivel de especie identificadas en este estudio, 166 coincidían con posibles patógenos humanos; v) El *Enterobacter cloacae* (56,45%) fue de las especies más dominantes seguido por la *Pseudomonas putida* (6,07%), el *Fusobacterium ulcerans* (3,08%) y el *Acidaminococcus fermentans* (2,03%) respectivamente. La *Aeromonas hydrophila*, la *Klebsiella pneumoniae* y la *Pantoea agglomerans* constituían a las especies menos dominantes. Este estudio apunta al efecto catastrófico en la salud pública y el medio ambiente que puede resultar del tratamiento conjunto de aguas servidas de hospital con las mismas que son domésticas en plantas de tratamiento de aguas servidas municipales y a el uso de los lodos resultantes en la agricultura, el cuál es un método común de eliminación de lodos y se practica en países en vías de desarrollo.

Palabras clave: lodo de hospital, Secuenciación de Nueva Generación, patógenos nosocomiales, patógenos emergentes, patógenos raros.

INTRODUCTION

Nosocomial infections cause significant mortality and financial losses each year. According to WHO, nosocomial infections account for 2 million cases and approximately 80,000 deaths each year. The endemic burden of nosocomial infections is significantly higher in low and middle income countries than in high income countries. Neonates are specifically more prone to nosocomial infections with infection rate ranging from 3-20 times in developing countries than developed countries (Nejad *et al*. 2011). The identification of potential reservoirs for nosocomial pathogens and their proper monitoring can help to alleviate the global burden of nosocomial infections to a great extent.

Hospitals are ecological niches for nosocomial pathogens. Recent studies suggest that environmental reservoirs like wastewater sources play a major role in the transmission of nosocomial pathogens within a hospital setting (Lensing *et al*. 2018). However published data on the role of wastewater treatment systems particularly its byproduct in transmitting nosocomial pathogens outside the hospital environment is still lacking. Sludge is a byproduct of a wastewater treatment system. Of the various microbial processes beneficial to the crucial task of wastewater treatment, adsorption constitutes a major mechanism by which pathogens are removed from the effluents by adhering to activated sludge flocs (Naughton and Rousselot 2017). Earlier studies have shown that several potential pathogens, such as *Leptospira, Arcobacter* and *Mycobacterium*, prefer attached states and either form biofilms or aggregate in activated sludge flocs (Ahmed *et al.* 2017, Pereira *et al*. 2017, Fang *et al.* 2018). Considering this argument, it can be hypothesized that hospital sludge can act as a potential reservoir for nosocomial pathogens released from infected patients or wastewater sources present in a hospital environment. Further, hospital sludge may provide an environment where competitive selective pressures and horizontal gene transfer can act as driving force for the emergence of novel pathogens; therefore, we hypothesize that it can act as a reservoir for not only well known but also several new or previously overlooked pathogens.

Though culturable methods have played a pioneering role in enhancing our knowledge about the infectivity of hospital sludge, these methods are limited by the fact that the majority of environmental bacteria are unculturable. Further, the limited number and range of pathogens that could be detected by culturable methods combined with the ability of some water-related pathogens to find niches in water systems like formation of biofilms or the ability of some pathogens to enter the viable but non-culturable state are some of the technical

limitations of the culture based approach that explains the paucity of quantitative information regarding the infective risk posed by hospital sludge (Felfoldi *et al.* 2010, Ramírez *et al.* 2015)

Recently established NGS technologies such as illumina have significantly improved the investigation of environmental samples by identifying the uncultivable and low abundance microorganisms which are otherwise difficult to detect by traditional molecular tools (Kumaraswamy *et al.* 2014). Past NGS studies have reported that biodiversity of activated sludge (AS) is greater than that of influent wastewater (Lee *et al.* 2015), also uncultivated species are more abundant in various types of AS (Liaw *et al.* 2010, Tomazetto and Oliveira 2013). Importantly, there is a lack in systematic monitoring of hospital sludge pathogenic microbiota using this technique.

Thus, the aim of the present study was to study the pathogenic diversity (as number of species) of hospital sludge focusing mainly on human pathogens that are not traditionally studied in wastewater management by the use of NGS approach. The present study will help to re-evaluate the infective risk of untreated hospital sludge and also define its role in vectoring pathogens to municipal wastewater treatment plants (WWTPs) in cases where hospital wastewater is co-treated with domestic wastewater.

MATERIALS AND METHODS

Study site and sampling

In this study, sludge samples were collected from a hospital wastewater treatment plant (HWWTP) located in Jaipur (Rajasthan). The HWWTP is based on membrane bioreactor technology and has a capacity of 400KLD. A schematic diagram of the HWWTP is given in Figure 1. Grab samples of hospital sludge were collected in sterile glass containers in the month of June 2017. All the samples were stored at -20˚ C until further use.

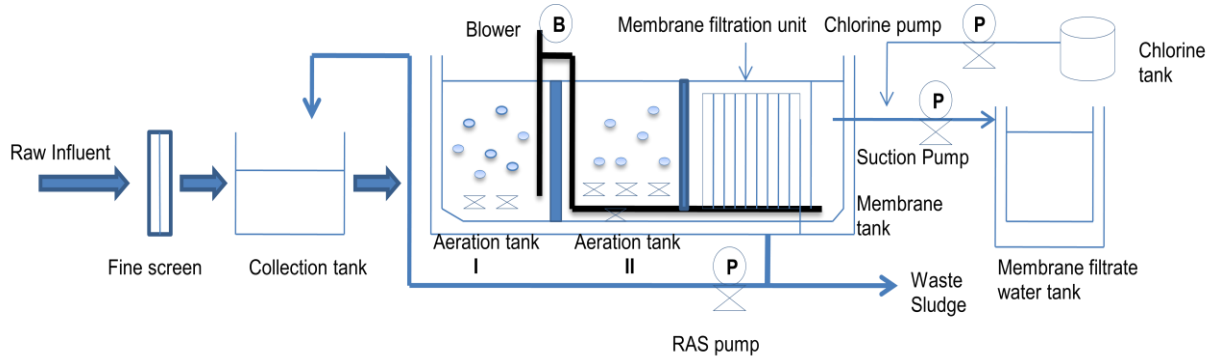

Figure 1: Schematic Diagram of the hospital wastewater treatment plant

Next Generation sequencing of hospital sludge

Metagenomic DNA of the composite sludge sample was extracted using the XcelGen soil DNA kit (Xcelris Genomics, Xcelris Labs Limited, Ahmedabad, India). The extracted DNA was stored at -20˚C prior to sequencing using the Illumina Miseq platform.

The V3-V4 hyper-variable regions of 16S rRNA gene were amplified using universal primers (forward primer 5'CCTACGGGNBGCASCAG 3' and reverse primer 5'GACTACNVGGGTATCTAATCC 3'). The PCR reactions were conducted using the following program: denaturation at 98˚C for 5 min, 27 cycles of 30s at 95˚C, primer annealing at 55˚C for 30s, elongation (30s) at 72˚C and final extension at 72˚C ˚C for 5min. The amplified product was purified using XcelGen DNA Gel Purification Miniprep Kit. The 16S rRNA gene library for paired-end illumina sequencing was constructed using Nextera XT Index Kit according to the manufacturer's protocol. The final amplicon libraries

were purified using 1X AMpureXP beads (Beckman Coulter). Purified amplicons were loaded onto Illumina Platform for cluster pair-ended sequencing according to the manufacturer's instructions.

Bioinformatics Analysis

The raw sequences were filtered by removing barcodes and primer sequences. Low quality sequences having phred scores less than 25, mismatched primers, or sequences shorter than 100 base pairs were removed. The filtered FASTQ files were uploaded to MG RAST server (Meyer *et al.* 2008). A 97% similarity index was used to carry out taxonomic assignment within the SILVA reference database. The abundance data for bacteria at different taxonomic levels were considered for further downstream analysis. Figure 2 shows the flowchart of the methodology used for the present study.

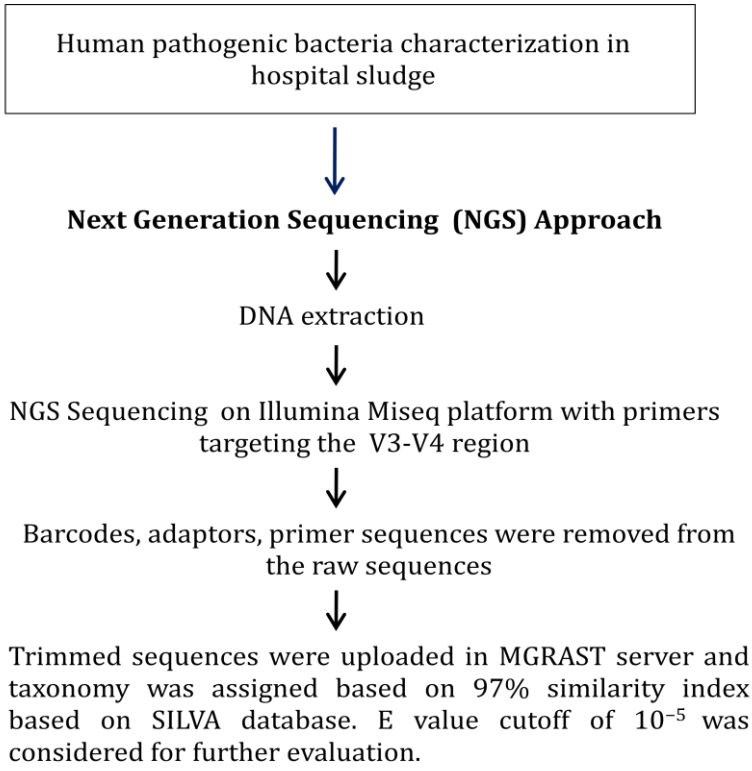

Figure 2: A flowchart of methodology used for studying the pathogenic diversity of hospital sludge by NGS approach

RESULTS

Sequencing results

Illumina sequencing of hospital sludge generated a total of 3, 78,026 number of paired- end reads. After trimming, sorting and quality control, the data set included 2, 78, 848 reads with an average length of more than 100 base pairs. It was clustered into 580 species-level OTUs, 378 of which included more than one read as shown in the Electronic Supplementary Material (ESM) 1. Since the present study aimed to look at fine diversity or low abundance pathogenic species, singleton OTUs were not discarded as we assume they may be close enough to a biological sequence and thus may be informative in downstream analysis also, some pathogenic species may only be present as a single read. Singleton OTUs comprised 0.077% of the reads that suggest an acceptable quality of

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sequencing and initial data filtering stages. Uncultured bacteria (not classified to any phylum) represented nearly 13.95% of the reads. Shannon index of 4.42 and rarefaction curve approaching plateau (as shown in figure 3) both indicate that highly diverse microbial communities were present in hospital sludge.

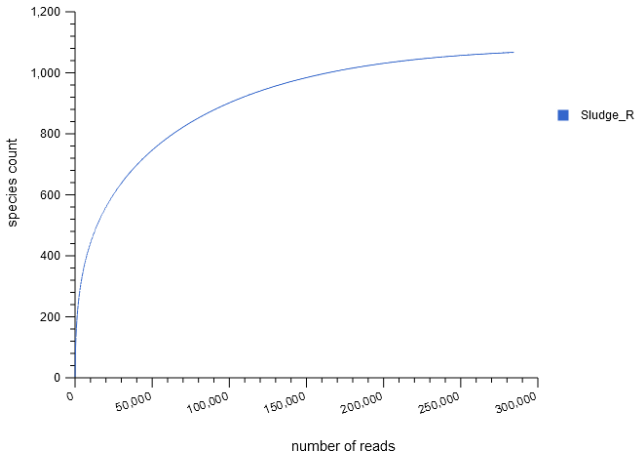

Figure 3: Rarefaction analysis for hospital sludge sample

Figure 4 shows the phylum abundance analysis of the hospital sludge samples. Proteobacteria (average abundance-70.53%) was found to be the most dominant phylum. Several studies have identified Proteobacteria as a possible microbial signature of disease (Rizzatti et al. 2017). Other dominant phyla included Firmicutes (5.74%), Actinobacteria (3.79%), Fusobacteria (3.19%) and Bacteroidetes (2.11%). All these phyla except Fusobacteria are reported to dominate AS sample (Zhang *et al*. 2012). Other phyla with an average abundance of less than 0.5% such as Planctomycetes, Verrucomicrobia, Synergistetes, Tenericutes, Chloroflexi, Spirochaetes, Acidobacteria, Cyanobacteria, Deferribacteres, Chlorobi, Nitrospirae, Lentisphaerae were profiled as rare phyla.

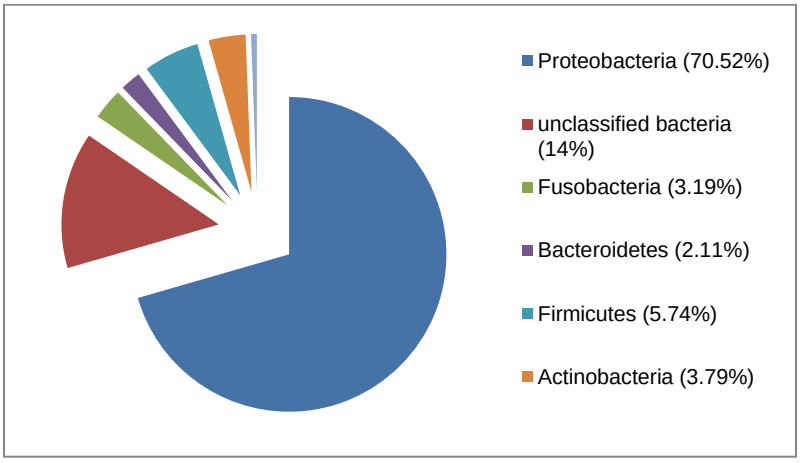

Figure 4: Phylum abundance analysis of hospital sludge. Minor phyla less than 0.5% of the total sequences are grouped as 'Rare phyla'

Within Proteobacteria, Gamma-Proteobacteria (68.9%) dominated the hospital sludge followed by beta (0.66%), alpha (0.83%) and epsilon-subdivisions respectively as shown in Figure 5. Among the different classes of Proteobacteria, the maximum unculturable species belonged to the class Beta-Proteobacteria (0.60%) followed by gamma (0.36%), alpha (0.08%) and delta (0.01%) subdivisions. Members of the family Enterobacteriaceae dominated the sludge sample occupying approximately 58.14% of the sequence reads (Figure 6).

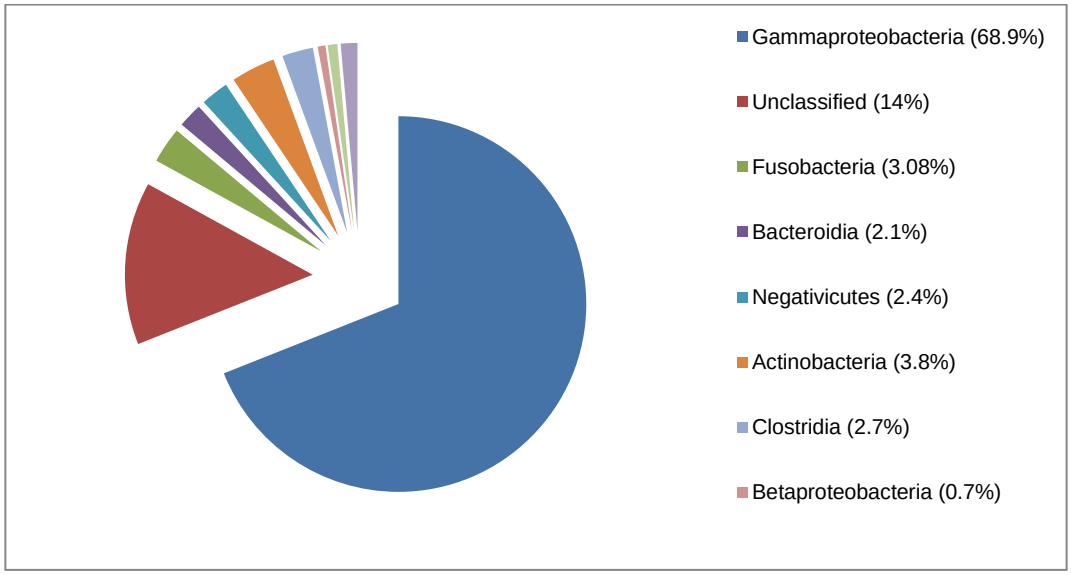

Figure 5: Class abundance analysis of hospital sludge. Minor classes less than 0.5% of the total sequences are grouped as 'Others'

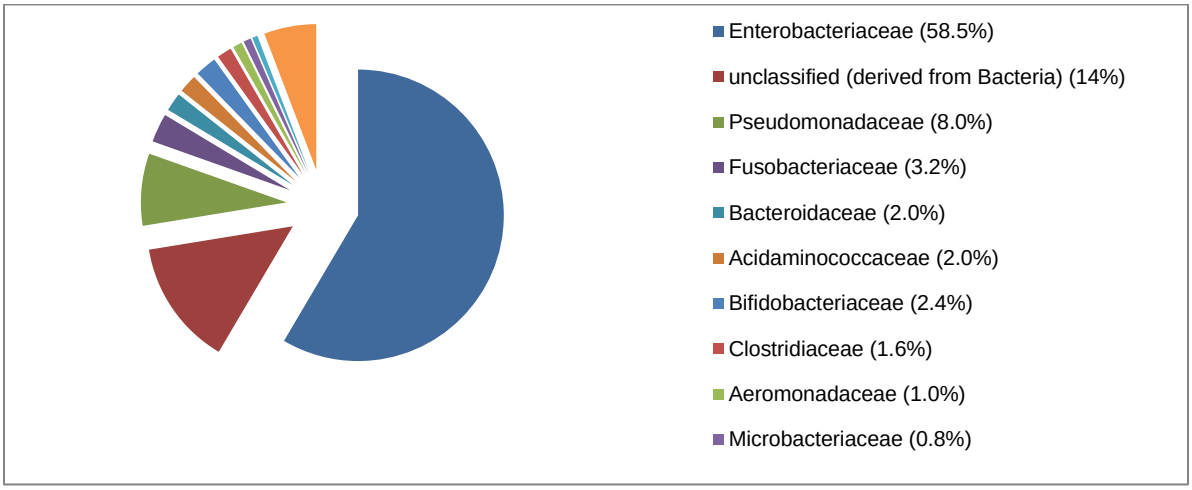

Figure 6: Family abundance analysis of hospital sludge. Minor families less than 0.5% of the total sequences are grouped as 'Others'

Pathogenic diversity

An analysis of the pathogenic profile of the hospital sludge revealed that 72.35% of the reads matched with bacterial species reported to be pathogenic to humans. These pathogenic sequences are affiliated with 81

genera and 166 species-rank OTUs as shown in Electronic Supplementary Material (ESM) 2. *Enterobacter cloacae* dominated the sludge sample by representing more than half (56.45%) of the sequence reads, followed by *Pseudomonas putida* (6.07%), *Fusobacterium ulcerans* (3.08%) *Acidaminococcus fermentans* (2.03%) respectively. *Aeromonas hydrophila*, *Klebsiella pneumoniae* and *Pantoea agglomerans* represented other less dominant species (average abundance <1 but >0.5). The remaining 3.56 % of the reads comprised the low abundant microbial species and are enlisted in Online Resource 2

Notable among the low abundant group were certain rare and emerging pathogens. Rare pathogens were the ones with ≤ 10 reported cases in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/). This group included some rarely described nosocomial pathogens belonging to genus *Microbacterium* and *Corynebacterium* (e.g *M.chocolatum, M. schleiferi, M. aurum, C. mucifacien, C.imitans, C. kroppenstedtii, C. ureicelerivorans*) among others like *Peptoniphilus harei, Butyricimonas virosa, Actinomadura crimea, Anaerotruncus colihominis, Pannonibacter phragmitetus* (Seng *et al.* 2013).

More than 70% of the species identified in this study are reported to be associated with hospital acquired infections indicating that nosocomial pathogens were the dominant force shaping the hospital sludge bacterial profile.

DISCUSSION

The objective of the present study was to study the pathogenic profile of hospital using the recent NGS approach to estimate the potential risk associated with its disposal. Several emerging as well as rare human pathogenic bacteria were identified in hospital sludge using Illumina Miseq sequencing platform. The NGS data support our hypothesis that hospital sludge can act as a reservoir for several potential pathogens that are not traditionally studied and thus could play a larger role in the spread of these pathogens than estimated. A similar study conducted by Vincenti *et al.* (2014) also reported that water sources within the hospital environment can be a potential source for not only known but also other emerging pathogens.

The presence of nearly 14% of the reads as unculturable bacteria indicates new or unknown bacteria that might be present in hospital sludge further emphasizing the need to explore this complex yet microbiologically diverse habitat.

An important finding of the present study was that the majority of the bacteria identified in this study are nosocomial pathogens. Bacteria are responsible for about 90% of nosocomial infections compared to other microbes (Gatermann *et al.* 2005). Though nosocomial infections can be caused by any bacteria, there is a rising trend of these infections being caused by multidrug resistant (MDR) opportunistic pathogens. According to WHO (2017), MDR bacteria found in hospitals, nursing homes and among patients dependent on devices like catheters and ventilators form the most critical group to be included in the global list of priority pathogens. These include *Acinetobacter, Pseudomonas* and various members of family Enterobacteriaceae. Fatal infections caused by MDR pathogens have become an emerging concern worldwide.

Enterobacter cloacae were the most dominant species identified in this study. *E.cloacae* forms part of the MDR *Enterobacter cloacae* complex (ECC complex). The ECC complex is associated with a wide variety of human infections and has emerged as one of the leading causes of nosocomial infections worldwide specifically owing to their resistance to the last-resort carbapenems (Annavajhala *et al.* 2019, Monahan *et al.* 2019). Other members of the ECC complex like *E. asburiae, E . hormaechei , E. cancerogenus* and *E. aerogenes* identified in this study formed part of the low abundant microbiota.

Next in the list of dominating species was *Pseudomonas putida*. Health-care infections caused by *P.putida* are rare and mostly reported in case of immunocompromised patients (Kim *et al.* 2012, Erol *et al.* 2014). Clinical data on *P. putida* infections is scarce probably due to its rarity, relatively low virulence, and higher antimicrobial susceptibility compared to *P. aeruginosa* (Carpenter *et al.* 2008, Yoshino *et al.* 2011). The recent emergence of MDR and carbapenem-resistant *P. putida* has raised a global concern for this bacterium (Almuzara *et al.* 2007,

Bennett *et al.* 2009). Besides *P.putida*, other rarely pathogenic non-aeruginosa pseudomonads like *P.pseudoalcaligenes, P.alcaligenes, P.stutzeri, P. fulva, P.monteilii and P.fluorescens* were also identified in this study as part of the low abundant microbiota.

Last in the list of dominating pathogens were the Gram-negative anaerobes *Fusobacterium ulcerans* and *Acidaminococcus fermentans* respectively. *F. ulcerans,* has been isolated from tropical ulcers and has not been reported in AS samples till date. *A. fermentans* has often been isolated from mixed cultures in the past. Few studies even suggested its role as an opportunistic pathogen in humans (Liu 2011). The identification of this bacterium in hospital sludge indicates the growing incidence of infections caused by this bacterium.

Other members of family Enterobacteriaceae, implicated as important nosocomial pathogens identified as part of the low abundant microbiota in this study included a) the opportunistic foodborne pathogens like *Salmonella spp*, *Yersinia enterocolitica* and *Cronobacter spp. Cronobacter* causes rare but life threatening infections in neonates and immunocompromised infants (Zeng *et al.* 2019). *C. sakazakii, C. malonaticus, and C. dubliniensis* are three prevalent species of this genus that were identified in this study. b) *Buttiauxella agrestis* - a rare human pathogen reported to cause rare post cesarean surgical site infections. There are no previous reports of the identification of this organism from the hospital environment (Antonello *et al.* 2014) c) *Kluyvera cryocrescens* - another potentially dangerous pathogen causing rare infections in humans, previously reported to have been isolated from hospital sinks and sewage (Yoshino *et al.* 2016) d) Others such as *Morganella morganii, Citrobacter spp, and Hafnia alvei* .

Gammaproteobacteria dominated the hospital sludge sample unlike municipal sludge where Betaproteobacteria formed the dominant class (Zhang *et al.* 2012). Gammaproteobacteria is the most diverse class of gram negative bacteria which includes a number of human pathogens while Beta-Proteobacteria contains only a few pathogens of clinical relevance. Except for a few rare opportunistic pathogens like *Bordetella avium and Oligella ureolytica*, most of the other pathogenic species belonging to Beta-Proteobacteria identified in this study are implicated as emerging nosocomial pathogens.

Some of the clinically important species within the class Gammaproteobacteria identified in this study included:

a) *Aeromonas spp.* that are gaining importance worldwide as emerging pathogens of concern in sludge and water distribution systems owing to their inherent capability to form biofilms and their resistance to chlorination (Igbinosa *et al.* 2012) *Aeromonas* species are linked with human diseases such as gastroenteritis, septicemia, muscle infections and skin diseases (Janda and Abbott 2010). Aeromonads were reported in 6.5% of patients in India (Sinha *et al.* 2004). Among the various *Aeromonas spp.* identified in this study, *A.hydrophila* was the most frequent isolate.

b) *Shewanella spp* that included *S.putrefaciens* and *S.algae*. Most cases of *Shewanella* infections are reported from areas with warm climate and the bacteria are often isolated from polymicrobial infections. *Shewanella spp* are rarely pathogenic and rare cases of their association with medical devices or causing hospital-associated infections and outbreaks have been reported in literature (Vignier *et al.* 2013).

c) Nearly six species belonging to the genera *Acinetobacter* were identified in this study of which *A. haemolyticus* and *A.calcoaceticus* were the more frequent isolates. High environmental tenacity, resistance to desiccation and high frequency of antibiotic resistance favors the transmission of this organism. *Acinetobacter spp.* may serve as an important environmental reservoir for resistant elements that transform into clinically compatible strains. *Acinetobacter* also shows a high frequency of an extreme drug resistant (XDR) phenotype. The hallmark of XDR phenotype is resistance to carbapenem antibiotics (Wong *et al.* 2017)

Pseudomonas aeruginosa, Acinetobacter calcoaceticus-baumannii complex and Stenotrophomonas maltophilia form part of the non-fermenting G-ve bacilli (NFGNB) group. NFGNB have emerged as one of the most common causes of nosocomial infections due to their resistance to a vast array of antimicrobials (Enoch *et al.*

2007). Water environments have been reported to play an important role in the development and spread of resistance in NFGNB (Baquero *et al.* 2008). Other members of this group identified in this study included *Shewanella putrefaciens*, *Burkholderia cepacia, Ralstonia pickettii* and *Alcaligenes faecalis.* Reports suggest that emerging opportunistic NFGNB e.g *R.pickettii* and *S. maltophilia* due to their higher antibiotic resistance could be potentially more dangerous than the other well known waterborne pathogens isolated from hospital environments (Vincenti *et al.* 2014). *Burkholderia cepacia* complex (BCC) constitutes another rarely reported NFGNB especially in India owing to poor laboratory identification of this organism (Gautam *et al.* 2011). BCC is ranked fourth worldwide after *P. aeruginosa, A.calcoaceticus-baumannii complex* and *S. maltophilia*. However, Postgraduate Institute of Medical Education and Research (PGIMER), ranked BCC as the third most common nonfermenter (Gautam *et al.* 2011).

Rare pathogens identified in this study signify the clinical burden resulting from the emergence of new bacterial pathogens.

Anaerobes constituted another important group of pathogens identified in this study. Anaerobic bacteria predominate skin and mucous membranes (Murphy and Frick 2013), are fastidious, and mostly isolated from polymicrobial infections with other known pathogens, therefore, often overlooked. They are generally considered to be relatively low virulent opportunistic pathogens. Infections caused by these organisms can be life-threatening. Clinically important anaerobic genera identified in this study included G-ve anaerobic bacilli (GNAB) like *Bacteroides, Prevotella ,Porphyromonas Fusobacterium* and *Leptotrichia* , G-ve anaerobic cocci (GNAC) like *Veillonella and Acidaminococcus,* Gram +ve anaerobic bacilli (GPAB), including spore formers like Clostridium and non-spore formers like *Bifidobacterium, Actinomyces, Anaerotruncus, Eggerthella, Eubacterium, lactobacillus, Propionibacterium* and also recently added genera like Atopobium and Mobiluncus and lastly gram +ve anaerobic cocci (GPAC) like *Finegoldia,Peptoniphilus, Peptostreptococcus* (Shenoy *et al.* 2017). Nearly 12 species belonging to the genus *Clostridium* were identified in this study, most of which are reported as uncommon or occasional human pathogens.

Another important group of nosocomial pathogens identified in this study were the aerobic non-sporulating, pleomorphic G+ve bacilli known as Diphtheroids. Diphtheroids are commensals of the skin and mucous membranes and are traditionally considered as mere contaminants when isolated from clinical samples. However, their increasing antibiotic resistance and association with nosocomial infections have made them clinically more significant in recent years (Chandran *et al.* 2016). Diphtheroids cause opportunistic infections in immunocompromised patients especially those depending on indwelling devices or those having a long stay in hospitals or nursing homes. Nosocomial strains of Diphtheroids can survive in the form of biofilms in hospitals causing multidrug-resistant infections (Chandran *et al.* 2016). The medically relevant diphtheroids identified in this study included the genera *Corynebacterium* (non-diphtheriae), *Brevibacterium, Microbacterium, Rothia, Arthrobacter, Leifsonia aquatica*, *Propionibacteriu. acnes, Rothia* species (*R.mucilaginosa and R.dentocariosa*).

Human body maintains a delicate mutualistic equilibrium with the commensal microflora present in it. Sometimes, this delicate equilibrium gets disturbed, in which case some commensal bacteria that did not evolve as pathogens may simply take on this role as a consequence of the inability of the host to maintain homeostasis (Ehrlich *et al.* 2008). Since hospitals contain a different subset of human population that is more susceptible to infections due to their compromised state of health, the probability of finding such commensals turn pathogens becomes high in case of hospital patients. The finding of several commensals of relatively low virulence in hospital sludge support the above argument and our hypothesis that hospital sludge is an underestimated reservoir for new pathogens.

Lastly, nearly 66 bacterial pathogens identified in this study were not tabulated by Woolhouse and Sequeria (Woolhouse and Sequeria 2005). Their pathogenic role was identified in subsequent years by various scientific studies. Most of these pathogens fall in the category of either rare or emerging pathogens. This

observation further supports our study that constant monitoring of environmental samples can provide new insights into the pathogenic diversity of these samples.

CONCLUSIONS

In conclusion, hospital sludge can act as an important environmental reservoir for several emerging as well as rare nosocomial pathogens and could play a major role in transmitting these pathogens outside the hospital environment. Most nosocomial infections get noticed only when they become epidemic. Therefore a proper monitoring and surveillance of environmental reservoirs will help to gauge any risk that might incur by the release of either classical or emerging nosocomial pathogens that in turn might help in the timely prevention of any epidemic caused due to such pathogens. The study further throws light on the health hazards that might result by the direct disposal of hospital sludge without proper treatment or the disposal of mixed sludges generated during the co-treatment of hospital wastewater with domestic wastewater that is often the case observed in developing countries. The latter sludges if utilized as fertilizers on agricultural land which is anecdotally the most practiced method of sludge disposal in developing countries might have catastrophic consequences on public health. Lastly, the study will help policy makers to redefine current standards and guidelines for hospital sludge disposal in an environmentally safe manner.

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Conflicts of interest: The authors declare that they have no conflict of interest

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