Original Research Article

Prevalence of carbapenem-resistant bugs in rectal swabs from patients/working staff in Medical Intensive Care Units**.**

Abstract

Introduction

With increased exposure to antibiotics, changes in the endogenous microbiota due to constant pressure of antibiotic selection can happen silently, resulting in the culmination of multidrug resistant strains. As the normal microflora is commonly implicated in human infections, these resistant strains can lead to nosocomial infections or a limited outbreak. This study was undertaken to investigate the carriage of carbapenem-resistant isolates in the rectal swabs of patients admitted in Medical Intensive Care Units (MICU) of our hospital.

Methods

Between December 2016-february 2017, rectal swab samples from 178 patients and 31 staff members of MICU were collected, isolates identified and tested for carbapenemase activity. Patients who were detected with carbapenemase activity in rectal swabs were further analyzed for carbapenemase activity in clinical samples. The activity of isolates (if any) was co-related with the clinical samples in patients and real time PCR (Stratagene) was carried out for genotype analysis.

Results

Of the 209 strains obtained from srectal swabs, 29 (13.8%) isolates from patients and none from staff demonstrated carbapenemase activity. Thirteen o~~O~~ut of these 29~~ Thirteen~~ patients were carriers and none of their clinical samples showed any growth. Twelve patients showed similarity i.e. rectal swabs and clinical samples showed similar type of Gram negative isolates with carbapenemase activity. No correlation was observed in 4 patients. Real time PCR analysis for *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-beta-lactamase 1 (NDM) genes in twelve patients showing similar isolates has shown that none of the isolates tested positive for KPC whereas nine isolate pairs showed NDM gene expression indicating endogenous infections.

Conclusion

Screening for carriage of carbapenem resistant enteric Gram negative bacteria in patients undergoing elective or emergency gastrointestinal surgical procedures can guide clinicians

about the antibiotic choices as these groups of patients are at high risk of possible endogenous infections.

Key words: Healthcare associated infections, Carbapenem resistance, Multi-drug resistance, Intensive care unit.

Introduction

Nosocomial' or 'healthcare associated infections' (HCAI) are classified as infections which manifest in the patient post 48h of hospital admission and are absent at the time of admission (1). Such ~~kind of infections occur~~kind of infections occurs when the patient is availing the healthcare facilities and may sometimes occur even after patient discharge. Moreover, nosocomial infections also comprise the occupational infections that occur among the medical staff (1). Nosocomial infections are mainly caused by diagnostic or therapeutic interventional procedures including catheters and ventilators, and are also influenced by other factors including patient's immune system, underlying illness, antibiotic exposure, hospital environment and bacteriological flora prevalent within a hospital (2). It has been reported that 7% of hospitalized patients in developed and 10% in developing countries can acquire one of the HCAI (3). The patients in Intensive Care Units (ICUs), surgical units, neonates and undergoing organ transplant are comparatively at higher risk of undergoing HCAIs (1). The rate of HCAIs in ICU patients has been predicted as high as 51% according to Extended Prevalence of Infection in Intensive Care (EPIC II) study (3).

Bacteria are the most common pathogens responsible for nosocomial infections and the pathogen source can be exogenous or endogenous. 10% of the pathogens causing HAIs arise from endogenous microflora of the patient (4). The patient becomes vulnerable to infections by its own endogenous natural flora when the immune system of the patients becomes very weak because of underlying illness and the continuous antibiotic exposure. The infections which arise mainly from patient's own microflora are surgical site infections and catheter associated urinary tract infections (CAUTIs) (5)(6). These pathogens cause infections if they travel to other body parts from the parts where they are usually found. With increasing exposure to antibiotics, the endogenous microbiota faces constant pressure of antibiotic selection and can result in the emergence of multidrug resistant endogenous strains. These multi-drug resistant nosocomial infections are associated with prolonged hospital stay, socio-economic implications, increased morbidity and mortality rates (7). This surveillance study

Comment [EM1]: Replace with (5-6).

was undertaken to investigate the carriage of carbapenem-resistant isolates in the rectal swabs among patients admitted in MICU in our hospital.

Materials and methods

Study design

This study was conducted on patients suffering from different bacterial infections and admitted to our hospital for treatment during the period between Dec. 2016 to Feb. 2017. The study was conducted as per the ethical principles of Declaration of Helsinki.

Study population

We enrolled 209 patients in this study. ~~The number of patients included in the study were 209.~~ Specimens were collected from 301 subjects from MICU including the hospitalized patients and the staff. Rectal swabs (perirectal swabs were not allowed) were collected from all the subjects and immediately transported to the lab. Cotton swabs were used for collecting rectal samples and were done by inserting and rotating the 1cm swab into the rectum. Following sampling, the swab was placed in the tube which contained 1ml sterile transport medium and the tube was vortexed for 1 min at maximum speed in the lab. All 209 rectal swab specimens were subjected to analysis. Clinical samples were obtained and analyzed only from the patients who were detected with carbapenemase producing isolates in rectal swabs. 29 pairs of carbapenemase producing rectal swabs were analyzed in parallel with the clinical isolates using qPCR-based analysis.

Culture-based quantification of carbapenem resistant isolates

Viable bacterial counts were performed for transport medium containing bacteria (100µl) by serial 10-fold dilutions in 0.9% saline. This was followed by plating of inoculum on tryptose soy agar plates supplemented with 5% sheep blood to quantitate the total culturable aerobic bacteria (TAB), and plating on CHROMagar KPC plates (HyLabs, Rehovot, Israel) for determining carbapenem resistant bacteria (CRB). After 18h of growth at 37°C, viable bacterial counts were done and the CRB to TAB ratio (CFU/ml) was determined.

Comment [EM2]: 209 patients and the rest were personel? Please define.

Comment [EM3]: Please provide the method of identification of the bacterial isolates. You only describe the culturing method and the method to identify the production of KPC carbapenemase.

Quantitative real-time PCR

qPCR was performed for analyzing the KPC or NDM producers using KPC and NDMspecific primers and miScript-SYBR Green Mix (Qiagen).

Comment [EM4]: Please provide the primers used for identification or proper references of this method. You previously described the identification of KPC carbapenemase with chromAgar plates. How many samples were analyzed by qPCR?

Results

Demographic characters

A total of 301 subjects from MICU including the patients suffering from different infections and the working staff were screened for this study. Of the 309 subjects screened, rectal swabs were collected from 209 subjects who consisted of 31 members from staff and 178 patients. Overall, the number of males (n=113, 54%) were more compared to females (n=96, 46%). The mean age was 47 ranging from 27 to 75 years. The common co-morbidities associated with patients at the time of hospitalization were hypertension (n=54, 24%), diabetes mellitus (n=46, 22%) and chronic liver disorders (n=19, 9%). 34.8% (62/178) of the patients were diagnosed with hospital acquired infections (HAIs). 27 patients had acquired HAIs inside MICU and 35 had acquired in wards which were shifted to MICU.

In vitro susceptibility analysis

Culture susceptibility analysis has shown that twenty-nine (13.8%) isolates demonstrated carbapenemase activity among 209 screened rectal swab isolates. All the rectal swab samples from the staff had susceptible microbiota and carbapenem resistance was not reported in any of the isolates. All the 29 (13.8%) carbapenemase producing isolates were from the patients included in the study. The isolates with carbapenemase activity in descending order are *E. coli* (17), *K. pneumoniae* (7), *P aeruginosa* (3) and *A. baumannii* (2).

Comment [EM5]: Which method did you use? Kirby-Bauer or MIC? Please refer the method in Material and Methods. Did you use CLSI or Eucast breakpoints? Please also define.

Comment [EM6]: Please define which type of carbapenemase was detected. A. baumanii carbapenem resistance is related to OXA rather than KPC or NDM, whereas Pseudomonas is connected with NDM carbapenemase. Klebsiellae has shown both NDM, KPC and OXA.

Only 29 patients who were detected with carbapenemase activity in rectal swabs were further analyzed for carbapenemase activity in clinical samples. The clinical samples obtained from these 29 patients and processed further were urine (16), sputum (8), fluid (3) and blood (2). Analysis of rectal swabs and clinical samples have shown that thirteen out of 29 patients were carriers as isolates were obtained from rectal swabs only and none of the clinical samples showed any growth. 16 patients showed growth in both rectal swabs and clinical samples. Of the 16 patients showing growth, twelve patients showed similarity i.e. rectal swabs and clinical samples showed similar isolates. The isolates from rectal swabs

were different from the clinical samples in four patients. For details of the isolates reported in rectal swabs and clinical samples and their source specimens, refer to Table 2 and 3.

Genotype analysis

Real time PCR (Stratagene) was carried out for the amplification of NDM and KPC genes. Out of the 12 samples showing similar isolates in rectal swabs and clinical samples, 10 pairs revived and all of them tested negative for KPC. 09 pairs tested positive for NDM gene in both rectal swab and clinical samples proving that patient had endogenous infection in these 9 patients. Only 1 pair showed dis-similar results as rectal swab tested negative for NDM whereas clinical sample tested positive for NDM.

Comment [EM7]: Please provide information for each group (what bacteria were NDM positive, and KPC positive).

Discussion

The human gastrointestinal tract serves as an endogenous reservoir of pathogens for various opportunistic pathogens causing various infections including urinary tract infections (UTI) and nosocomial infections like skin & soft tissue infections (SSIs) (1). However, with increased consumption of antibiotics, intestinal microflora faces constant pressure of antibiotic selection, which has resulted in the emergence of multidrug resistant endogenous strains. Carbapenems are the most effective modified beta-lactam antibiotics with a broad spectrum of activity against these multi-drug resistant strains (8) (9). Their unique molecular structure which combines carbapenem with the beta-lactam ring confers them exceptional stability against most beta-lactamases including AmpC (ampicillin and carbenicillin) and extended spectrum beta-lactamases (ESBLs) (9). Being highly effective against wide range of bacterial species, carbapenems are considered the most reliable last-resort treatment for bacterial infections. However, the excessive carbapenem prescription has led to the emergence and spread of carbapenem-resistant strains and created a global public health crisis (10) (11).

This widespread carbapenem resistance has not even spared the normal microbiota of patients. In our study, 16.3% of the isolates from the rectal flora of the admitted patients in MICU demonstrated carbapenemase activity. Rai *et al.* (12) has reported that 9.9% isolates demonstrated carbapenemase activity in a prospective surveillance study undertaken to investigate the carriage of carbapenem-resistance among 242 screened *Enterobacteriaceae* isolates in the gastrointestinal tract among patients attending the outpatient clinic in a tertiary care center of East Delhi, India. Similarly, Das *et al.* (13) has reported that neonates with endogenous Gram negative bacilli in the gut had a higher

incidence of clinical sepsis than those without. Moreover, in 50% of cases, the genotypes of the organisms found in the blood were indistinguishable from their gut counterpart.

The similar carbapenem-resistant organisms identified in clinical and rectal swabs from 31% of patients show that the opportunistic endogenous flora translocate to the ideal and favorable surfaces and turn pathogenic taking advantage of suppressed host immune system, mucosal barrier permeability, stress or co-morbidities, in vivo devices, surgical procedures, etc. Moreover, the kind of patient flora can hint at the antimicrobial susceptibility profile of patient and guide physicians about the antibiotic choices. Out of 29, 13 patients were also carriers of carbapenem-resistant isolates. This resistant endogenous flora may remain silent for months in the gut of the carrier without leading to any visible symptoms or translocate to other favorable surfaces, become opportunistic and induce healthcare-associated infections, or may lead to limited outbreaks through cross-transmission to other individuals (14) (4).

Conclusion

An important component of infection control program is active surveillance of drug resistant strains, including ESBL-producing strains and carbapenem-resistant strains. From this study, it can be concluded that more surveillance studies of this kind need to be performed for better understanding of the pattern of drug resistance among routine gut colonizers in Asian sub-continent. A thorough and continuous screening for drug-resistant isolates can help in formulating a better antibiotic policy for a hospital, particularly for patients admitted in ICUs and oncology units, being more vulnerable to opportunistic infections.

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Isolates	Number	%age
No. of rectal swabs in which CR isolates were identified	29/178	16.2 %
CR isolates identified in rectal swabs only	13/29	44.8%
Clinical samples and rectal swabs with similar CR isolates	12/29	41.3%
Clinical samples and rectal swabs with different CR isolates	4/29	13.7%

***CR-** carbapenem resistant

Table 1: Distribution pattern of carbapenem resistant isolates in rectal swabs and clinical samples of different patients.

Name of organism from rectal swab	No.	Name of organism (Clinical sample)
CRE	01	CRK (Urine)
CRK	01	CRA (Urine)
CRE	01	CRE (Urine)
CRE	01	CRK (Sputum)

CR- carbapenem resistant
CRE- carbapenem resistant *E.coli*
CRK- carbapenem resistant *K. pnuemoniae*
CRA- carbapenem resistant *A. baumannii*

Table 2: Distribution pattern of dissimilar isolates in rectal swabs and clinical samples.

Name of organism isolated from rectal swab and clinical sample	No.	Clinical sample
CRE	06	4 from urine & 1 each from Sputum & Fluid
CRK	04	2 from Urine & 2 from Sputum
CRP	01	Sputum
CRA	01	Blood

CRE- carbapenem resistant *E.coli*
CRK- carbapenem resistant *K. pnuemoniae*
CRP- carbapenem resistant *P. aeruginosa*
CRA- carbapenem resistant *A. baumannii*

Table 3: Distribution pattern of similar isolates in rectal swabs and clinical samples.