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Point Prevalence Survey for Screening of Carbapeneme Resistant Enterobacteriaceae in ICU Patients using CDC Protocol and Chromogenic Agar

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A B S T R A C T

Carbapenamse Resistant *Enterobacteriaceae* (CRE) have disseminated widely since being first reported in 2001. CRE often carry genes that confer high levels of resistance to many other antimicrobials. So they are usually resistant to all β-lactam agents as well as most other classes of antimicrobial agents. The treatment options for patients infected with CRE are very limited. Healthcare-associated outbreaks of CRE have been reported. The aim of this work is to know the prevalence of CRE *Escherichia coli* and *Klebsiella pneumoniae* in hospitalized high-risk ICU patients using chromogenic agar and CDC laboratory protocol for CRE detection, in order to implement infection control measures efficiently in order to prevent emergence of hospital-based outbreaks of carbapenemase producing organisms in a tertiary care university hospital. This point prevalence study was conducted in two weeks duration. Fifty four stool specimens were collected from ICU patients. CRE were detected in 38 out of 54 (70.4%) of isolates by chromogenic agar and CDC laboratory protocol for CRE detection. Out of 38 isolates 32 (59.2%) were positive for carbapenemase activity by ertapenam susceptibility and MHT. Chromogenic CRE agar and meropenem susceptibility had a perfect agreement in detecting all resistance mechanism for carbapenems and ertapenam susceptibility and MHT had a perfect agreement in detecting carpabenemse producing *Enterobacteriaceae.*

Introduction

Carbapenems (imipenem, meropenem, ertapenem and doripenem) are often the drugs of last resort for bacteria producing extended spectrum beta-lactamases (ESBL) in the *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae*. Especially, with increasingly occurring resistant to quinolones, aminoglycosides, trimethoprim-sulfamethoxazole and other antibiotics. Siegel *et al.*, 2006). Carbapenem-resistant *Enterobacteriaceae* (CRE) have disseminated widely since being first reported in 2001 and are usually resistant to all β-lactam agents as well as most other classes of antimicrobial agents. Carbapenem-resistant *Enterobacteriaceae* often carry genes that confer high levels of resistance to many other antimicrobials. The treatment options for patients infected with CRE are very limited. Healthcare-associated outbreaks of CRE have been reported. Patients colonized with CRE are thought to be a source of transmission in the healthcare setting. Carbapenem-resistant *Enterobacteriaceae* have been associated with high mortality rates up to 40 to 50% in some studies (Calfee and Jenkins, 2008). Carbapenemases are specific β-lactamses with the ability to hydrolyze carbapenems and

sometimes other classes of β-lactams. These carbapenem-hydrolysing enzymes are a cause of grave concern in all five Gram-negative members of the so-called 'ESKAPEE' (*E. faecium, S. aureus, Klebsiella, Acinetobacter, Pseudomonas, Enterobacter, E. coli*) pathogens, which collectively cause the majority of healthcare-associated infections. This concern is warranted because there are too few active antibiotics left on the formulary shelf, and the developmental pipeline of new agents now offers little more than a trickle. A key challenge for the next decade is to limit the spread of carbapenemase producers and so minimize their impact on public health (Woodford and Nordmann, 2012).

Early identification of carbapenemase producers in clinical infections is therefore mandatory to prevent development of those hospital-based outbreaks. This process involves culturing patients who might not be epidemiologically linked to known CRE patients but who meet certain pre- specified criteria. This could include everyone admitted to the facility, pre-specified high-risk patients (e.g., those admitted from long-term care facilities), and/or patients admitted to high-risk settings (e.g., intensive care units). Identifying patients who are colonized with CRE and placing these patients in isolation precautions may be an important step in preventing transmission (Siegel *et al.*, 2006).

Screening is used to identify unrecognized CRE colonization among hospitalized patients. Generally, this screening involves stool, rectal, or peri-rectal cultures and sometimes cultures of wounds or urine (If a urinary catheter is present). A laboratory protocol for evaluating rectal or peri-rectal swabs for CRE is recommended by CDC using Modified Hodge Test. However, it is important to note that this procedure has only been validated for *E. coli* and *Klebsiella* spp. CRE screening of hospitalized patients is a primary prevention strategy for all healthcare facilities either with CRE outbreaks or facilities that do not or only rarely admit patients with CRE infection or colonization. This intervention is applicable to both acute and long-term care settings (CDC, 2012).

The aim of this work is to know the prevalence of CRE *Escherichia coli* and *Klebsiella pneumoniae* in hospitalized high-risk ICU patients using chromogenic agar and CDC laboratory protocol for CRE detection, in order to implement infection control measures efficiently in order to prevent emergence of hospital-based outbreaks of carbapenemase producing organisms.

Materials and Methods

The current study was designed as point prevalence study in two weeks duration (1-15 September 2013) for screening of CRO in ICU patients following CDC Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenemase-Producing, *Klebsiella spp.* and *E. coli* (CDC, 2012). Fifty four stool samples were collected from patients fulfilling the inclusion criteria. Patients admitted for a long period in the hospital, patients admitted from long term care facilities, patients with overuse of antibacterial drugs and immuno-compromised patients admitted to intensive care units in Ain Shams University Hospitals [geriatrics (ICU. 7), neurological (ICU. 5), intermediate care unit (5), general medicine (ICU. 8), general surgery (ICU. 5), pediatric (ICU. 4), burn (ICU. 5), cardiac (ICU. 9) and bone marrow transplantation unit (6)]. Specimens were processed in the main Microbiology Laboratory in Ain Shams University Hospitals.

Screening medium

Stool samples were screened for the presence of carbapeneme resistant organisms (CRO) by

culturing on chromogenic CRE agar. (LIOFILCHEM, Italy). For stool samples, approximately 0.5 g of stool sample was suspended in 1 ml of 0.85% saline, and after vortexinga loop was used to inoculate the Chromogenic CRE agar. Chromogenic CRE agar were incubated for 18–24 hoursat 35 ± 2ºC in ambient air. Chromogenic (CA) CRE agar were examined for colony growth and color formation at 20–24 hours and again at 44–48 hours for any slower-growing colonies. Blue colonies on CA are associated primarily with carbapenemase-producing *K. pneumoniae*, but other species of Enterobacteriaceae also produced this color. Red colonies on CA were associated with carbapenemase-producing *E. coli*.

CDC laboratory protocol for detection of CRE

Stool samples were also processed according to CDC Laboratory Protocol for Detection of Carbapenem Resistant or Carbapenemase-Producing, *Klebsiella spp.* and *E. coli* on 3 days sequence (CDC, 2012) as follows:

Day 1

Tryptic soy broth (Oxoid, Uk) contains 10-µg Ertapenem and Meropenem disk (Oxoid, UK) each in a separate tube was prepared aseptically. Then one loopful of stool samples was inoculated into the broth. Tubes were incubated overnight at 35 ± 2ºC in ambient air.

Day 2

From each tube 100 µl of the incubated broth was subcultured on MacConkey agar (Oxoid, UK) and the plates were incubated for 24 hours at 35 ± 2ºC in ambient air. MAC was examined for the presence of slow to rapid lactose-fermenting gram-negative bacilli. The isolates were identified by using the API 20E system (bioMerieux, France).

Day 3

Modified Hodge test – this phenotypic test could be used to determine if reduced susceptibility to carbapenems is mediated by a carbapenemase. A dilution of 0.5 McFarland of the indicator strain. (*E. coli* ATCC 25922) was prepared in 5 ml of broth or saline. Dilution 1:10 was made by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of saline. A lawn of the 1:10 dilution of *E. coli* ATCC 25922 was streaked to a Mueller Hinton agar plate then allowed to dry for 3–5 minutes. Meropenem (10 µg) disks were placed in the center of the plate. In a straight line, test organism was streaked from the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate with one Meropenem disk.

After 16–24 hours of incubation, the plates were examined for a clover-leaf type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the Meropenem disk.

Modified Hodge test (MHT) – Positive test has a clover-leaflike indentation of the *E.coli* 25922 growing along the test organism growth streak within the disk diffusion zone (photo 1). MHT Negative test has no growth of the *E. coli* 25922 along the test organism growth streak within the disk diffusion zone (photo 2).

The carbapenem disks that are used in this procedure were quality control tested using disk diffusion methods and quality control strains as described in the CLSI guideline.

The ability to recover CRE using this procedure was assessed as follows: five mL of TSB containing the 10-µg carbapenem disk was inoculated with a swab that was used to sample a known CRE-negative stool specimen. In addition, the TSB was

inoculated with 0.5 mL of a 1 x 105 CFU/mL suspension of a known carbapenemase-producing isolate (e.g., *K. pneumoniae* ATCC BAA-1705). These inoculated TSB were processed as the protocol. The carbapenemase-producing *K. pneumoniae* should be recovered on the MacConkey agar.

For testing of specificity of the procedure, a carbapenem susceptible Klebsiella pneumoniae (e.g. ATCC 700603) was used following the same steps. The carbapenem susceptible *K. pneumoniae* isolate should not grow on the MacConkey agar.

Statistical Analysis

Descriptive statistics: Frequency and percentage of non numerical data were done. Kappa test were done using computer software program.

Results and Discussion

Fifty four stool samples were collected from patients fulfilling the inclusion criteria admitted to intensive care units in Ain Shams University Hospitals in two weeks duration. Out of 54 stool samples 29 were from females and 25 were from males. There is no statistical significance between gender and having CRE P value = 0.723.

The age of the participants in the study varied from 10 months to 76 years old. There is no statistical significance between age and having CRE P value = 0.762.

The duration of the length of stay varied from 1 day to 51 days with a mean of 9 days, considering 3 days or more as a risk factor to gain CRE. There is highly statistical significance between duration of length of stay and having CRE P value = 0.000. Carbapenem-resistant *Enterobacteriaceae* were detected in 85.7% of samples in Geriatrics ICU, in 80% of samples in neurological, general surgery and burn ICUs and in 75% of samples in general medicine and pediatric ICUs.

There were 34 patients having chronic medical condition. There was high statistical significance between having chronic medical condition and having CRE P value = 0.000.

Out of 54 participants in the study 45 (83.3%) patients were taking antibiotic, 35 (92%) out of 39 patients that were having CRE were taking antibiotic. There is high statistical significance between taking antibiotic and having CRE P value = 0.008).

Screening medium

Out of 54 stools specimens, 39 specimens were positive for CRE isolates by chromogenic agar. All *E. coli* isolates 23 out of 23 (100%) grown on chromogenic CRE agar gave a red colonies, 16 out of 16 (100%) *Klebsiella* isolates gave a blue violet colonies, 3(100%) out of 3 Enterobacter gave a blue-green colonies. There was a perfect agreement between results of Meropenem and chromogenic agar (Kappa= 1.000). There was a good agreement between results of Ertapenem and chromogenic agar (Kappa= 0.690). Chromogenic agar was faster for providing the data within 24 h for isolating of CRE colonized patients.

CDC Laboratory Protocol for Detection of CRE

On doing the CDC Laboratory Protocol for Detection of CRE, out of 54 stool specimens, 39 (72.2%) stool specimens contained Carbapenem-resistant *Enterobacteriaceae* (CRE) five (9.2%) stool specimens contained other non *Enterobacteriaceae* isolates. Ten stool samples were negative for any growth. Out of 39 stool specimens, 23 (59%) were *E.*

coli, 16 (41%) were *Klebsiella*, 3 (7.6%) were *Enterobacter*. Other non *Enterobacteriaceae* isolates were present as follows: six (13.6%) isolates were *pseudomonas.* three isolates only were pure growth and other three were mixed with other *Enterobacteriaceae*), four (9.0%) isolates were *Acinetobacter* (two isolates only were pure growth).

On comparing susceptibility results of meropenem and ertapenem for detection of carbapenem-resistant isolates, meropenem detected 44 out of 44 (100%) of all CRO and 39 out of 39 (100%) of CRE but Ertapenem detected 32 out of 39 (84.20%) of CRE isolates only, there were statistical significance between them (p = 0.03). On doing MHT in day three according to CDC Laboratory Protocol, six isolates of 39 (15.8%) of isolates were negative whereas 32 of 38 (84.2%) isolates were positive for carbapenemase activity. Modified Hodge test (MHT) was positive in 18 (78%) out of 23 *E. coli* isolates, 14 (87.5%) out of 16 *Klebsiella* isolates, three (50%) of *pseudomonasspp* and two (50%) out of four *Acinetobacter* isolates. There was a good agreement between Meropenem susceptibility and MHT (*Kappa*= 0.690) but there was a perfect agreement between Ertapenam susceptibility and MHT (Kappa= 1.000). According to Ertapenam susceptibility and MHT; the prevalence of carbapenemase producing *Enterobacteriaceae* is 32 out of 54 (59.2%).

-Stool samples that were positive for CRE or carbapenemase-producing Enterobacteriaceae were reported to the appropriate infection control personnel. Contact Precautions were recommended for all colonized patients by CRE

The global dissemination of Enterobacteriaceae harboring carbapenemases is a major public health concern. Surveillance and isolation have demonstrated effectiveness in reducing nosocomial acquisition of CPE. The Centers for Disease Control and Prevention (CDC) provided guidance on the isolation of carbapenemase producing *Escherichia coli* and *Klebsiella spp.* From rectal swabs by advising the use of 5ml Trypticase soy broth (TSB) supplemented with a 10-µg ertapenem or meropenem disc followed by subculturing on MacConkey agar (Mathers *et al.*, 2014).

The aim of this work is to know the prevalence of CRE *Escherichia coli* and *Klebsiella pneumoniae* in hospitalized high-risk ICU patients using chromogenic agar and CDC laboratory protocol for CRE detection, in order to implement infection control measures efficiently in order to prevent emergence of hospital-based outbreaks of carbapenemase producing organisms in a tertiary care university hospital.

In the current study, 54 stool samples were collected from patients fulfilling the inclusion criteria admitted to intensive care units (geriatrics ICU, neurological ICU, intermediate care unit, general medicine ICU, general surgery ICU, pediatric ICU, burn ICU, cardiac ICU and bone marrow unit) in two weeks' time as a point prevalence study. Stool samples were processed as CDC protocol 2012.

In the present study, Out of 54 stool samples, the carbapenem resistant positive isolates were 38(70.4%), 21(55.3%) were from females and 17(44.7%) were from males which contained the carbapenem resistant isolates. There is no statistical significance between gender and having CRE (P> 0.05). The age of the participants in the study varied from 10 months to 76 years old. There is no statistical significance between age and having CRE (P >0.05). Similarly, in studies by Haji *et al*., (2012) and Patel *et al.*, (2012) they tested 244, 99 strains respectively for carbapenemase production, and found that there was no significant difference as regards

age or sex. However, Lin and coworkers (2013) reported that carbapenem resistant isolates were more in the elderly (median age, 60 years).

In the current study, the length of stay varied from 1 day to 51 days with a mean of 9 days, considering 3 days or more as a risk factor to gain CRE. There is highly statistical significance between long duration of admission and having CRE P value = 0.000. Similarly, in Patel *et al.,* (2012) study, they found that a longer length of stay in hospital was highly significant with positive cases of carbapenamase producing isolates; especially in ICUs. In addition to that, Lin *et al.,* (2013) measured the prevalence range of carbapenamase producing isolates in long-term acute care hospitals, it was 10% -54% versus 0%-29% in short stay hospitals. This was also reported by Jocelyn *et al.,* (2012).

In the current study, there were 34 (92.1%) patients having chronic medical condition. There was high statistical significance between having chronic medical condition and having CRE P value = 0.000. In Jocelyn *et al.,* (2012) study, they reported that patient group colonized by CRE was more likely to have chronic medical conditions than control group. Also, Schwaber *et al.,* (2013) reported that chronic medical conditions which were significantly associated with CRE were liver disease, malignancy and immunosuppression. In the current study, out of 54 participants in the study 45 (83.3%) patients were taking antibiotic, 35 (92%) out of 38 patients that were having CRE were taking antibiotic.

There is high statistical significance between taking antibiotic and having CRE P value =. 0.008).Similarly, Patel *et al.,* (2012), reported that 76% of patient group and 96% of control group who received antimicrobial therapy were colonoized by CRE. Also, Jocelyn *et al.,* (2012), Leanne *et al.,* (2013) and Schwaber *et al.,* (2013) reported that 91%, 86% and 73% of cases respectively receiving antibiotics were colonoized by CRE. In another study done in Japan 6.4% of healthy adults carried carbapenemase producing strains compared to 58.4% in Thailand, where antibiotics are available over the counter and without prescription.

Screening medium

In the current study, out of 54 stools specimens, 39(100%) specimens were positive for CRE isolates by chromogenic agar. There was a perfect agreement between results of meropenem and chromogenic agar (Kappa= 1.000) as both methods detect crabapeneme resistance by all mechanisms. There was a good agreement between results of ertapenem and chromogenic agar (Kappa= 0.690).

Similarly, Brocco *et al*., (2013) made a study to demonstrate the efficiency of Chromatic CRE intended as a screening step of KPC producing strains, they found that KPC-producing *K. pneumoniae* grew uninhibited on Chromatic CRE and yielded green or blue colonies (100% sensitivity). Poor growth/no growth/atypical colony color was evident with KPC-non producing Enterobacteriaceae (Brocco *et al*., 2013).

CDC laboratory protocol for detection of CRE

On doing the CDC Laboratory Protocol for Detection of CRE, out of 54 stool specimens, 39 (72.2%) stool specimens contained Carbapenem-resistant Enterobacteriaceae (CRE), five. (9.2%) stool specimens contained other non *Enterobacteriaceae* isolates. Ten stool samples were negative for any growth. Out of 39 stool specimens, 23 (59% were *E. coli*, 16. 41%) were *Klebsiella*, 3 (7.6%) were Enterobacter. Other non

Enterobacteriaceae isolates were present as follows: six (13.6%) isolates were *pseudomonas.* Three isolates only were pure growth and other three were mixed with other *Enterobacteriaceae*, four (9.0%) isolates were *Acinetobacter.* two isolates only were pure growth) (Table 1). Similarly, in a study in Egypt made by Othman *et al*., (2016), they tested100 rectal swabs that were subcultured on MacConkey agar supplemented with different types of carbapenems. They found that 62% of rectal swabs were positive for CRE. In another study Hasanin *et al*., (2014) conducted a survey in Egypt for one-year duration, a total of 152 samples (65%) out of 234 gram negative bacilli samples developed extensively drug resistant infection. This high rate of CRE in Egypt is attributed to over the counter antibiotic abuse, noncompliance with antimicrobial stewardships and infection control measures in hospitals. On the other hand, in a study made by Nair and Vas 2013 in India, they found the CRE prevalence rate was 12.26%, from which the majority of the isolates were detected in urine samples (46%). They found that although most of the CRE isolates were detected in patient samples from the wards 42% and the ICU 26%, a significant number of isolates was also detected from the OPD patients (19%).

Another study by Mathers *et al*., 2014, they tested 6,860 perirectal screens in 2012 in Virginia using the CDC protocol, 185 specimens (2.7%) representing 79 unique patients were positive for CPE by phenotype. The majority of positive results were for *K. pneumonia* (97; 52%), *E. cloacae* (39; 21%), and *C. freundii* (24; 13%). In a metanalysis study made by Xu *et al*., (2015), they searched PubMed and Embase databases to identify rates of prevalence of CRE in Asia, they found that the rates was still low during the study period with average resistance rates of 0.6% imipenem and 0.9% Meropenem. They added that resistance rates to imipenem and meropenem in Enterobacteriaceae exhibited stably escalating trend. This low rate may be attributed to applying strict infection control measures and adopting antimicrobial stewardships to lower the abuse of antimicrobials.

In the current study, on comparing susceptibility results of Meropenem and Ertapenem for detection of Carbapenem-resistant isolates, Meropenem detected 44 out of 44 (100%) of CRO and 39 out of 39 (100%) of CRE but Ertapenem detected 32 out of 39 (84.20%) of CRE isolates only, there were statistical significance between them ($p = 0.03$).All isolates resistant to ertapenem were positive by MHT and all isolates which were negative by MHT were susceptible to ertapenem. This means that Ertapenem was more sensitive than Meropenem in screening of the carbapenemase enzyme. Meropenem was more sensitive than ertapenem in screening of resistance by different methods (efflux pump, mutations that alter the expression function of porins and PBPs).

Similarly Shannon *et al.,* (2009) made a study on to determine the positive predictive value and specificity of ertapenem resistance for KPC detection in 2,696 Enterobacteriaceae isolates and confirmed the presence of the KPC enzyme by MHT and PCR. They found that positive predictive value and specificity of ertapenem resistance for KPC detection were 74% and 99.2%, respectively. Similarly in Othman *et al*., (2016) study they found that Mac. ETP showed the highest sensitivity for detection of KPC producing CRE 71%.

Photo.1 MHT positive with clover-leaf like indentation

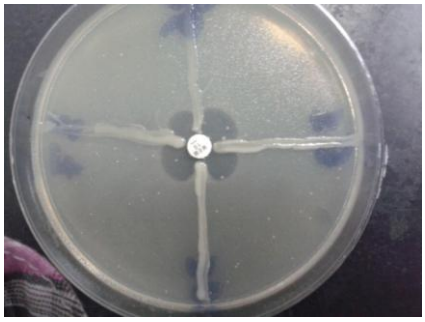
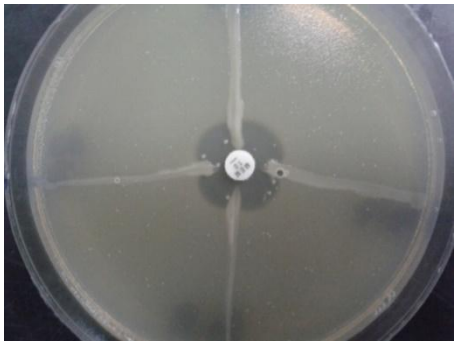


Photo.2 MHT negative with no clover-leaf like indentation



On doing MHT in day three according to CDC Laboratory Protocol, 32 out of 39 (84.2%) isolates were positive for carbapenemase activity whereas six isolates of 39 (15.8%) of isolates were negative which mean that these resistant isolates carry another mechanism of resistance to carbapenem. efflux pump, mutations that alter the expression function of porins and PBPs). There was a good agreement between Meropenem susceptibility and MHT (*Kappa*= 0.690) but there was a perfect agreement between Ertapenam susceptibility and MHT (Kappa= 1.000). Anderson *et al*., (2007) evaluated the modified Hodge test for detection of KPC-mediated resistance. The test demonstrated 100% sensitivity and specificity for detection of KPC activity.

In the current study, the prevalence of carbapenemase producing *Enterobacteriaceae* is 32 out of 54 (59.2%). Similarly, in a study by El Wartiti *et al.*, (2012), a total of 463 clinical isolates from rectal swabs were investigated over 19 months period at Microbiology Laboratory of Cheikh Zaid University Hospital in Rabat, Morocco. Carbapenemase producing isolates were 82.9% using the disk diffusion method and E-test, confirmed by MHT and PCR. Also, a voluntary surveillance program was done to assess the epidemiology of CPE in Ontario, by Public Health Ontario in collaboration with the Ministry of Health and Long-Term Care, the prevalence of carbapenamase producing *Enterobacteriaceae* (CPE) in large community hospitals in Canada was 59% when done on 458 isolates by PCR (PHO, 2013). Similarly in Othman *et al*., (2016) study, the prevalence of KPC producing CRE were 57%.

In Conclusion, CRE is a major global threat so early screening of colonized patients and isolating them is a crucial step to prevent outbreaks that could in hospital. Using

chromogenic CRE agar provides the data rapidly in order to implement infection control measures but may be unavailable for many limited resources laboratories. The CDC protocol is a well validated method which can be applied in every hospital especially with limited resources laboratories so as to screen for CRE and apply isolation efficiently.

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