

PREVALENCE OF CARBAPENEM RESISTANT ENTEROBACTERIACEAE INFECTIONS, THEIR MANAGEMENT AND OUTCOME AMONG CANCER PATIENTS.

PREVALENCE OF CARBAPENEM RESISTANT ENTEROBACTERIACEAE.

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ABSTRACT

Purpose: This study aims to evaluate the prevalence of CRE (Carbapenem-resistant Enterobacteriaceae) infections among cancer patients, their characterization, and follow-up of the patients to know the outcome.

Method: For one year from August 2018 to July 2019 samples of two hundred and forty-two non-duplicate clinical specimens were obtained from patients at our centre. Clinical and demographic data and clinical outcome are gathered from the electronic hospital medical records. Any significant growth from any source was processed for microbial identification and susceptibility by Vitek 2 Compact. Phenotypic confirmation of carbapenemase production was done as per CLSI (Clinical & Laboratory Standards Institute) guidelines using eCIM (EDTA-modified carbapenem inactivation method) and mCIM (modified carbapenem inactivation method).

Result: A total of 88 gram-negative isolates were isolated. *Escherichia coli* (53.4%) was the main Gram-negative isolate from all samples followed by *Pseudomonas aeruginosa* (21.5%). Among the Gram-negative Enterobacteriaceae (n=67) isolates, 31.3% were carbapenem-resistant. None was isolated from blood cultures. The average length of stay of CRE-infected patients was 10.33 days as compared to those not infected with CRE (5.4 days). The average cost of management per admission was significantly higher in patients with CRE infection.

Conclusion: This study provides a baseline data on the prevalence of CRE among people suffering from cancer in Central India along with its outcome, which might be helpful for better implementation of antimicrobial, diagnostic, and infection control stewardship.

Keywords - mCIM (modified carbapenem inactivation method), eCIM (EDTA- modified carbapenem inactivation method), Carbapenem resistant Enterobacteriaceae (CRE).

Introduction:

Cancer is a worldwide major cause of death, with over half occurring in developing nations (1). Leukaemia, breast cancer, stomach, colorectal, liver, and lung are the most frequent cause of cancer death (1).

Radiation, as well as chemotherapy in a cancer patient, may induce several changes in the body when cancer cells are destroyed by them. One important change is that such therapies decrease the immune system, which increases the risk you have of developing an infection, such as a bacterial infection. White blood cell count may reduce with radiation and chemotherapy generally called neutropenia. The range of bacterial infections may be moderate to risk of life-threatening. Therefore, it is necessary to pursue care to try to prevent extreme infections as quickly as possible.

Nowadays, Carbapenem resistance has been a significant challenge to public health, food protection, and economic growth. In every region of the globe, the ratio is raising to critically high. Carbapenems are beta-lactam antibiotics and are seen as the last phase in MDR (Multi-Drug Resistant) (such as doripenem, ertapenem, meropenem, and imipenem). In any nation, CRE may affect anyone of any age. An increasing number of infections in cancer patients

during chemotherapy treatments because they are immunocompromised patients including salmonellosis, tuberculosis, and pneumonia are getting difficult to heal. Resistance to antibiotics means longer stay in hospitals, higher treatment expenses, and higher mortality rates.

New pathways of resistance are evolving and growing worldwide and endanger our ability to cure common infectious diseases in cancer patients.

The problems in health care are growing due to MDR bacteria ("Multi-drug resistant"). The effectiveness impact of carbapenems, which are regarded as last-line therapies, is decreased by the increasing prevalence in gram-negative bacteria of different resistance mechanisms. The global spread of MDR pathogens reduces significantly the likelihood of successful antimicrobial care, particularly in a hospital environment (2). The occurrence of such high-resistant microorganisms makes an important contribution to long-term stay in hospitals and elevated death rates.

The emergence of MDR strains of Gram-negative bacteria that cause nosocomial infection has become a critical threat, specifically in cancer patients (3). The number of researches performed in cancer patients in the past few years is exclusively based on BSIs and the general incidence of Gram-negative bacteria was not studied (3). The goal of this research was therefore to assess the range and antibiotic resistance pattern of nosocomial infections of gram-negative bacteria among patients with Iranian cancer (4). It will help physicians choose suitable empirical treatments and have good epidemiological profiles to equate our case with other treatments (4).

Methods & Material:

Study Set-up: 170 bedded exclusive Cancer center in Central India.

Study period: One year (between August 2018 - July 2019)

Study design: Prospective cohort study

Inclusion criteria: This experiment involved samples from all cancer patients with suspected infection who were admitted to our hospital for one year from August 2018 - July 2019. All high risk cases e.g; Leukaemia, Colonic malignancy were screened for presence of CRE infection prior to initiation of therapy (Chemotherapy or surgery respectively)

Samples and data collection and Statistical analysis: Clinical non-duplicate specimens of patients suspected of Infection at any site (n=242) were obtained. From electronic hospital medical records, Type of malignancy, demographic data, site of infection, antibiotic treatment provided, length of stay and outcome was analyzed for statistical significance using Chi Square test using Epi Info software. P value <0.05 was considered as statistically significant.

CRE Screening test

In this study, CREs were detected from the stool specimens. A loop of stool (0.5 gm) was mixed in 4 ml of sterile saline and made as uniform suspension. 4 ml. TSB (Tryptic Soya Broth) was taken in clean glass test tube. Add 100 µl. of Stool Suspension in two separate test tubes and add a disc of Meropenem (10 µg) in one of it. After overnight incubation at 37 °C, subculture was performed from tubes with standard loops calibrated to 1 µl on MacConkey agar plates. The plates were incubated overnight and observed for growth of gram negative colonies. Any growth was identified by Vitek 2 compact as Enterobacteriaceae (30).

Bacterial Isolation and Susceptibility

The samples were inoculated on the MacConkey Agar (HiMedia, Mumbai), Tryptose Blood Agar base with 5% sheep blood (Bio smart, Mumbai), and Chocolate Agar (Bio smart, Mumbai) for 24 hours at 37°C temperature. If culture was negative, then plates were further re-incubated for the next 24 hours. Culture positive isolates were identified and susceptibility done by Vitek 2 compact. All Carbapenem-resistant Enterobacteriaceae were phenotypically confirmed with eCIM and mCIM test as per CLSI guidelines (5).

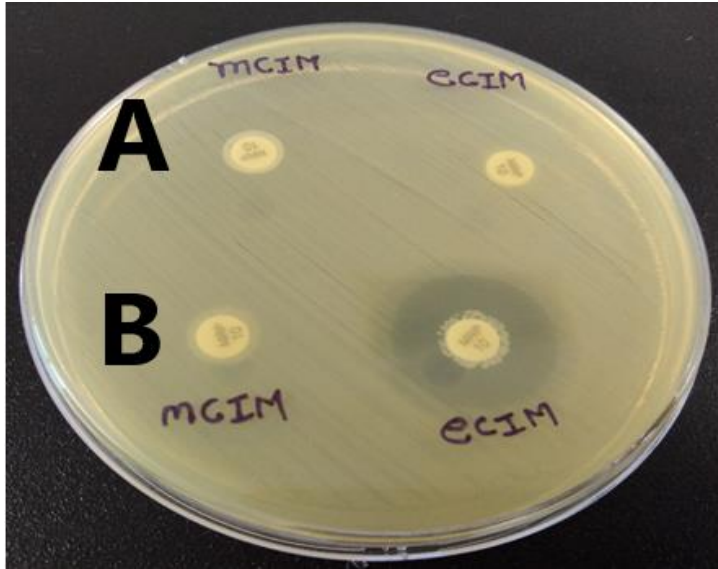
mCIM with eCIM test

A 1-µl loopful carbapenem-resistant Enterobacteriaceae group of bacteria were taken in two tubes containing 2 ml TSB. One tube was without presence of EDTA (mCIM), whereas in the other tube 20 µl of the 0.5M EDTA (eCIM). Meropenem disk (10 µg) was added to each tube and incubated at 35°C for 4 hrs ± 15 min. 0.5 McFarland of E. coli ATCC 25922 in the nutrient broth was used as control. After 4 hours the Meropenem disk was removed from the tubes and placed on freshly prepared M-H agar ("Mueller-Hinton") plates with a lawn culture of E. coli ATCC 25922. The plate was incubated for 18 to 24 hrs at 35°C in the ambient air. After 18 to 24 hours zone of inhibition was measured. Refer to figure no.1 for mCIM & eCIM interpretation.

Figure 1. mCIM & eCIM interpretation

A. Positive mCIM and Negative eCIM (serine carbapenemase)

B. Positive mCIM and eCIM (metallobetalactamase)



Result:

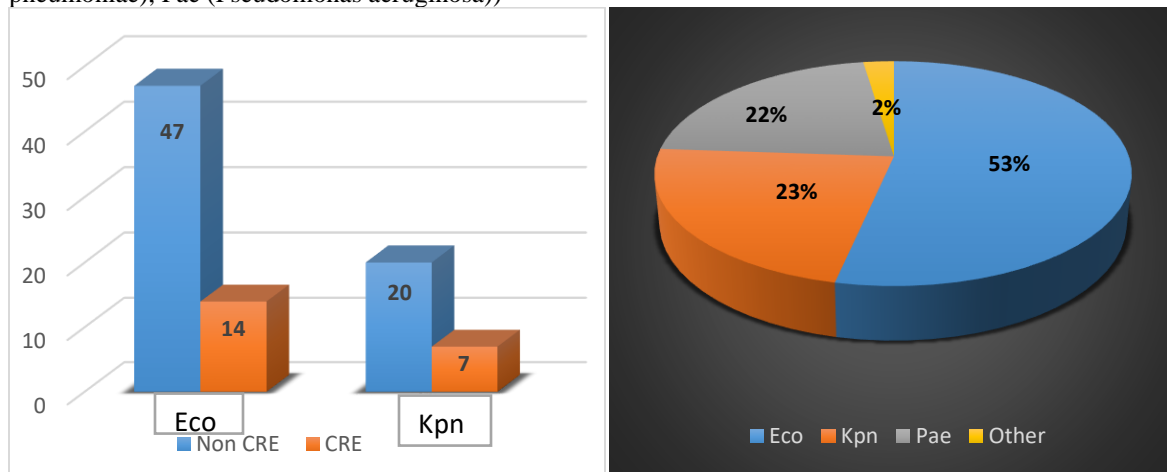
88 gram-negative bacilli have been isolated. Escherichia coli was the main isolated Gram-negative bacilli from all clinical specimens (53.4%) followed by Pseudomonas aeruginosa (21.5%). Among the Gram-negative Enterobacteriaceae (n=67) isolated, 31.3% were carbapenem-resistant refer to figure no. 2 Distribution of Gram-negative isolates and CREs. None was isolated from blood cultures. Refer to Table no.1 for the distribution of isolates from various samples. All screening

cultures for CRE were found to be negative.

Table no.1Distribution of isolates from various samples

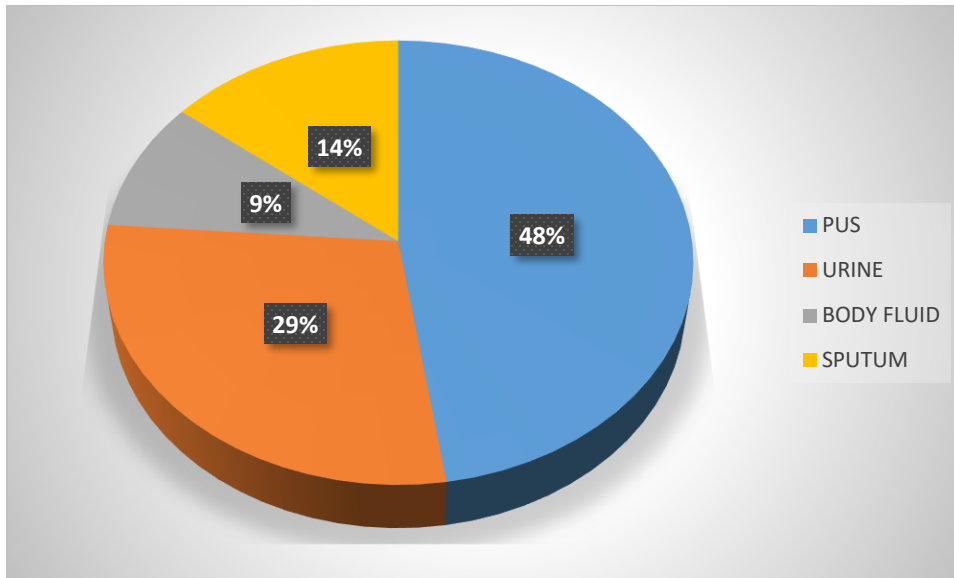
Culture sample	Pus	Blood	Urine	Body fluid	Sputum	Total
Total received	114	62	37	11	18	242
Positive isolates	78	14	18	8	9	127
Negative isolates	36	48	19	8	4	115
Gram negative isolates*	50	8	16	6	8	88
Enterobacteriaceae	34	6	16	3	8	67
CRE isolates#	10	0	6	2	3	21

Figure 2. Distribution of Gram-negative isolates and CREs [Eco (Escherichia coli), Kpn (Klebsiella pneumoniae), Pae (Pseudomonas aeruginosa)]



We have been isolated CRE from pus 48% (10/21), urine 29% (6/21), sputum 14% (3/21) and body fluid 9% (2/21). Refer to Figure no. 3 Isolate of CRE from different types of specimen in cancer patients.

Figure 3. Isolate of CRE from different types of specimen in cancer patients.



The average length of stay of CRE-infected patients was 10.33 days as compared to those not infected with CRE (5.4 days). The average cost of management per admission was significantly higher in patients with CRE infection. Refer to Table no.2 for the Clinical outcome for mean length of stay and cost of treatment in patients infected with or without CRE infections.

Table 2. Clinical outcome for mean length of stay and cost of treatment in patients infected with or without CRE infections.

Indicators	CRE	Non-CRE	P-Value
Mean Length of Stay	10.33	5.4	<.00001
Cost of treatment	131106.95	54153.76	<.002

In this study we have isolated CRE (n=21) from different types of cancer patients such as CA (carcinoma) head and neck 24% (5/21), CA gastrointestinal 29% (6/21), Leukaemia 19% (4/21), CA genitourinary 14% (3/21), CA breast 14% (3/21). Refer to Figure 4. Isolate of CRE from cancer patients. Three patients 14.2% (3/21) died within 28 days across all sites of the CRE infection. Refer to Table no 3. Distribution of first CRE isolates after diagnosis from different type of cancer patients.

Figure 4. Isolate of CRE from cancer patients.

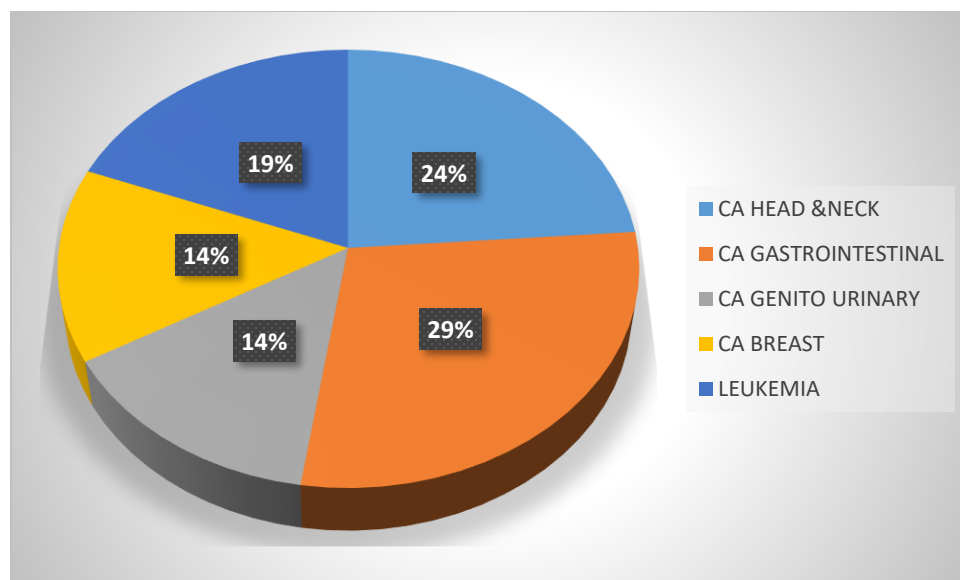


Table 3. Distribution of first CRE isolates after diagnosis from different type of cancer patients with 14 days and 30 days mortality and treatment given.

Types of cancer	Total isolates	E.coli	K. pneumonia	Mortality 14 days	Mortality 30 days	Treatment given
CA head & neck	5	2	3	-	-	Meropenem + Colistin
CA gastrointestinal	6	5	1	-	1	Tigecycline+ Colistin
CA genitourinary	3	2	1	-	1	Meropenem + Colistin
CA breast	3	1	2	-	-	Meropenem + Colistin
leukaemia	4	4	-	-	1	Meropenem + Colistin

Management of CRE infections

Source control along with combination therapy with either meropenem + Colistin/ meropenem+ Tigecycline/ Colistin+ Tigecycline were associated with favourable outcomes in case of CRE infections.

Statistical analysis:

The statistically relevant P-value <0.05 was generally considered. There was a considerable difference in length of stay <.00001 and the cost of treatment of patients infected with CRE was observed <.002 when compared to those infected with non-CRE organisms.

Discussion

Globally, the occurrence of CRE has appeared. The prevalence of CRE ranges somewhere between 13.95- 37.9% in India (6). The prevalence of CRE is more among Klebsiella pneumoniae (40% in our study). As with many other studies, metallobeta lactamase producers predominate the Indian scenario though sporadic cases of serine carbapenemase were reported. Only 2 (14.2%) of the Escherichia coli isolates in our study phenotypically identified as serine carbapenemase producers. Refer to Table no.4 for Isolate of CRE.

Table4. Isolate of CRE

Isolates	Total isolates	Serine carbapenemase mCIM + ve/ eCIM -ve	Metallobetalactamase mCIM + ve/ eCIM +ve
E. coli	14	2 (14.3%)	12 (85.7%)

K. pneumonia	7	-	7 (100%)
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Enterobacteriaceae are populations of the intestinal flora and important pathogens in both nosocomial and community settings. The present study illustrated the increasing prevalence of CRE from entire view of Asia, where data from each country also shows consistent trends. In mainland China according to the report of Mohanrin, the imipenem resistance of E.coli and K.pneumoniae in 2004-2005 is 0.0% - 0.7% (7), while the rate increase to 0.5% - 2.7% in 2010 (8). In Korea, the carbapenems resistance also follows such tendency. As the result of KONSAR (9), E.coli and K.pneumoniae is completely sensitive to imipenem in 2000, while in 2009, the resistance rate come to 0.1% - 0.5% respectively (10).

The generally prevalence of CRE among the 2 ICU patients was 9.8 which is lower than those of 12.2% reported in long- term care hospitals and acute- care hospitals in Japan (11) and 37.9% in Iran (12). Likewise, higher figures of 12% have been reported in post- acute care facilities ward in Israel (13) and 20.4% in the hematology- oncology wards in India (14). Our prevalence rate is higher than the reported figures of 6.6% in China (15) and 5.4% in USA while these studies were carried out in the ICU, medical surgical, and acute rehabilitation units (16). It is pertinent to point out that a direct comparison with these studies should be interpreted with caution as the settings and patients were different from ours. However, in studies, similar to ours, reported on hospitalized patients in Spain and France, the prevalence rates were 2.9% and 5.3% respectively (17, 18). Similarly, a prevalence of 3.8%, much lower than ours, was also reported in a large refractory nosocomial outbreak of KPC- producing E.coli in the central Manchester University Hospital UK (19) as well as in another study conducted in a large teaching hospital in Northern Italy, where the percentage of CRE rectal colonization dropped significantly from 0.2% - 0.05% after introduction of infection control interventions over a period of 30 months (20). It is important to note that only a few of these studies were conducted on ICU patients. In concordance with the low community- acquired colonization rates encountered in our study, a study in Spain did report a much lower community- acquired colonization of rate of 0.4% (17).

Kumarasami et al found 23.7% prevalence rate of CRE from isolates from Hariyana (21). 13% to 51% prevalence rate was noted by Wattal et al in a tertiary care hospital from Delhi (22). In a study in Mumbai, Nair et al found it around 12.26% (23). A range of 17 to 22% was observed by Gupta et al in a study in northern India (24). These factors and longer duration of hospital stay might have contributed for the high prevalence of CRE in the study (25). In India like developed countries non communicable disease, accidents and injury are becoming the leading cause for hospitalisation (26). Also, they are likely to get intervention as a part of indoor patient management, contributing to CRE infection. These include healthcare and long term care exposure, invasive devices, drains and endotracheal intubation (27). In the study of Nair et al 42% (23) and Singh et al 39.4% (28). In study by Nagaraj et al most carbapenemase producing isolates were from general surgery, general medicine, and ICU (29).

Conclusions:

Combined therapies are recognized only as a suitable and highly efficient option for treating CRE infections as we did in our research. In infection prevention of CRE infections, source control plays an important function. This baseline data on the prevalence of CRE among people suffering from cancer with its outcome might be helpful for better implementation of antimicrobial, diagnostic, and infection control stewardship at our Institution.

These results also argue the importance need to carry out screening test for carbapenem resistant bacteria before each admission of cancer patient.

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Sample Credit author statement

Dr. Manisa sahu: Conceptualization, Methodology, software, reviewing and editing

Rahul Kumar Banerjee: Data curation, writing- original draft preparation.

Dr. Varun dwivedi: Reviewing and editing, visualization, investigation, supervision.

Dr. Shreshtha Tiwari: visualization, investigation, validation.

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