INTRODUCTION

Enterobacteriaceae are one of the major groups of bacteria and community-acquired infections; these cause increased morbidity and mortality, especially with increased resistance rates [1]. Extended spectrum beta-lactamases (ESBLs) lead to multidrug resistance by transfer between bacteria and are seen in Enterobacteriaceae [2]. Carbapenems are important group of antibiotics used as a last option, especially in ESBL producing multidrug-resistant Enterobacteriaceae [3]. As a result of increased use of carbapenems, carbapenemases are widespread in Enterobacteriaceae family and particularly in Klebsiella pneumoniae; so effective treatment options are decreasing [2,4]. Carbapenemase-producing K. pneumoniae emerged in the late 1990s and has become a serious health problem worldwide [5].

Carbapenem-resistant K. pneumoniae (CRKP) can cause nosocomial infections and outbreaks with high mortality rates. Such infections occur mainly in patients admitted to intensive care units (ICU) with several underlying diseases and histories of having received prolonged courses of antibiotics [6]. Several resistance mechanisms have been described. Carbapenemase production (K. pneumoniae carbapenemase [KPC]), metallo-β-lactamase (MBL) and porin loss, combined with the overproduction of ESBL, are described as the most common mechanisms of carbapenem resistance [7,8].

Carbapenemases producing K. pneumoniae strains are considered endemic in some areas. For example, studies by the European Antimicrobial Resistance Surveillance (EARS-net) showed that the prevalence of carbapenem-resistant K. pneumoniae has increased from 1-2% to 15% in Italy in 2006-2009 [9]. Due to this resistance spreading rapidly around the world, there has been a need for new therapeutic agents. Polymyxin and colistin were out of use since the early 1970s due to its side effects of neurotoxicity and nephrotoxicity, but colistin has become a preferred antimicrobial agent with increase of infections with resistant Enterobacteriaceae [10-12]. However, excessive use of colistin has led to resistance to this drug [13]. The resistance to colistin seen in K. pneumoniae strains is reported to be due to reduced affinity of its side effects of neurotoxicity and nephrotoxicity, but colistin has become a preferred antimicrobial agent with increase of infections with resistant Enterobacteriaceae [10-12]. However, excessive use of colistin has led to resistance to this drug [13]. The resistance to colistin seen in K. pneumoniae strains is reported to be due to reduced affinity of

MATERIALS AND METHODS

The present study was carried out in a Tertiary Care Hospital of Karnataka, South India, with bed strength of 650. A total of 518 non-repetitive clinical isolates of K. pneumoniae were collected over a period of 3 years (2014-2016) from our ICUs, i.e. multidisciplinary ICU, neurosurgery ICU, intensive thoracic unit, neonatal ICU, pediatric ICU, coronary care unit, and renal ICU. These isolates were obtained from endotracheal aspirate (111), blood (80), urine (59), pus (22), bronchoalveolar lavage and sputum (21), catheter tips (15), fluids (13), and tissue specimens (8).

K. pneumoniae was identified using standard biochemical methods. Disk-diffusion tests were performed to determine susceptibility to antimicrobials, according to the Clinical and Laboratory Standards Institute (CLSI). Escherichia coli ATCC 25922 was used as a quality control. Isolates were screened for the ESBL phenotype by the standard double-disk synergy test, and KPC was screened both by evaluating breakpoints and using modified Hodge test, according to CLSI guidelines [14].
Imipenem plus ethylenediaminetetraacetic acid (EDTA) discs were used to detect the presence of MBL (HiMedia Laboratories, Mumbai). MICs of meropenem and colistin (Sigma-Aldrich Corporation, St. Louis, US) were determined by the agar dilution method according to the guidelines from the CLSI [14]. The colistin breakpoint was evaluated using breakpoints for Enterobacteriaceae recommended by the European Committee on Antibiotic Susceptibility Testing. (resistant: ≥2 ug/ml; sensitive: ≤2 ug/ml). K. pneumoniae ATCC 700603 was used as a quality control.

RESULTS
Totally 518 isolates of K. pneumoniae were isolated during the study, of which 329 (63.5%) isolates were CRKP isolates. The clinical source and distribution of K. pneumoniae in different ICUs are shown in Tables 1 and 2.

The antibiotic susceptibility of CRKP to other drugs is shown in Table 3. Antibiotic susceptibility testing revealed that very few antibiotics were susceptible.

With regard to colistin 91/329 (27.65%), isolates were resistant and the MIC ranged between 4 and ≥512 ug/ml.MIC90 and MIC50 of colistin were 16 and 4 ug/ml (Fig. 1).

In the present study, we found that 85/91 (93.4%) isolates that showed resistance to colistin were from MICU and resistance to carbapenems also seen in MICU (83.3%).

Table 1: Clinical sources of carbapenem-resistant Klebsiella pneumoniae (329)

| Clinical specimen        | N (%) |
|-------------------------|-------|
| Tracheal aspirate        | 111 (33.73) |
| Blood                   | 80 (24.3) |
| Urine                   | 59 (17.9) |
| Pus                     | 22 (6.7) |
| BAL and sputum           | 21 (6.4) |
| Catheter tips            | 15 (4.5) |
| Fluids                  | 13 (3.95) |
| Tissue                  | 8 (2.4) |

Table 2: Distribution of CRKP in ICUs

| ICU         | N (%) |
|-------------|-------|
| MICU        | 274 (83.3) |
| NSICU       | 25 (7.9) |
| ITU         | 11 (3.4) |
| NICU        | 8 (2.4) |
| PICU        | 5 (1.5) |
| CCU         | 3 (0.91) |
| RICU        | 2 (0.6) |

ICU: Intensive care unit, MICU: Multidisciplinary intensive care unit, NSICU: Neurosurgery intensive care unit, ITU: Intensive thoracic unit, NICU: Neonatal intensive care unit, PICU: Pediatric intensive care unit, CCU: Critical care unit, RICU: Renal intensive care unit, CRKP: Carbapenem-resistant K. pneumoniae

Table 3: Antibiotic susceptibility pattern of CRKP* (n=329)

| Antibiotic          | CRKP (%) susceptible |
|---------------------|----------------------|
| Cotrimoxazole       | 22 (6.6)             |
| Gentamicin          | 58 (17.6)            |
| Amikacin            | 59 (17.9)            |
| Netilmicin          | 42 (12.7)            |
| Tobramicin          | 19 (5.7)             |
| Ciprofloxacin       | 9 (2.7)              |
| Ofloxacine          | 10 (3.0)             |
| Levofloxacine       | 22 (6.6)             |

*All cephalosporins, betalactamase, and betalactamase inhibitors were resistant. CRKP: Carbapenem-resistant Klebsiella pneumoniae

Out of 329 carbapenem-resistant isolates, 216 were positive for modified-Hodge test and 59 K. pneumoniae were screened positive for imipenem/imipenem EDTA test. The rest of the 54 carbapenem-resistant isolates were negative for both tests.

DISCUSSION
K. pneumoniae is highly prevalent in hospitals and causes many nosocomial infections. The emergence of drug resistance in K. pneumoniae continues to be of critical concern for the choice of treatment options against infections caused by this bacterium.

Carbapenem resistance in Klebsiella spp. is an emerging problem and is a cause of concern as many nosocomial Klebsiella spp. are detected to be resistant to most other antibiotics.

Colistin is used as last resort of antimicrobials, especially in the present worrisome therapeutic scenario of multidrug resistant and pan drug-resistant Gram-negative infections. The drug acts on outer cell membrane of Gram-negative bacteria (GNB) and releases lipopolysaccharides [15]. This in turn results in disruption of cell membrane leading to leakage of cell content, causing cell lysis and finally cell death [16,17]. Within years after the reuse of colistin, there have been reports of colistin-resistant strains [18]. Indiscriminate antibiotic use in India is leading to cases of bacteria resistant to colistin.

ICUs are the epicenter for spawning multidrug resistance within hospitals. Many patients are transferred to the ICU from other healthcare facilities, where they have acquired resistant pathogens. Patients within the ICU undergo invasive procedures, treatment with antibiotic.

Combinations and exposure to other patients with resistant pathogens [19]. Multiple mechanisms exist for ICU pathogens to acquire antibiotic resistance. These mechanisms include enzymatic inhibition of drugs, alteration of proteins targeted by antibiotics, changes in metabolic pathways, antibiotic efflux, alterations in porin channels, and changes of membrane permeability [20].

In the present study, we found 63.5% (329/518) K. pneumoniae was multidrug resistant. The majority of the carbapenem resistant isolates were isolated from tracheal aspirate (33.73%), followed by blood (24.3%) and urine (17.9%). Gupta et al. found resistance to meropenem (22.16%) was more compared to imipenem (17.32%) in his study from different bacteria.

He also found that a significantly high resistance was seen in ICU patients, 37.3% and 31.9% for meropenem and imipenem, respectively [21]. El-Mahdy et al. in his work on cancer patients found only 4% of them were resistant to carbapenems [22]. Bashir et al. in his research study found 27.9% isolates were carbapenem-resistant [23].

Dizbay et al. [24] analyzed nosocomial infections produced by carbapenem-resistant Klebsiella spp. in ICU and their risk factors. They found that carbapenem resistance is significantly high in ICUs and more.
frequent (78.57%), and resistance was more often seen in respiratory tract specimens.

In the current study, all the isolates were screened for MIC of colistin. The prevalence of colistin resistance in K. pneumo niae from ICUs was (91/329) 27.65%, and the MIC ranged between 4 and >512 ug/ml.

Taneja et al. in a study from North India found 16% of the carbapenem-resistant strains were resistant to both tigecycline and colistin [25]. Giani et al. in Italy conducted a nationwide cross-sectional survey on CRE, carried out in mid-2011, the overall percentage of colistin resistance among KPC-KP was found to be 22.4% [26]. In another study in Italy Capone et al. observed 36.1% resistance to colistin in GRKP [18]. In a recent study by Kontopidou et al., 51 (34%) patients were colonized by pathogens with an intrinsic resistance to colistin [15].

Ghafur et al. reported a series of 13 patients with colistin resistance from South India [27].

With the increased emergence and spread of GRKP, treatment options are decreasing. As a reserve agent, colistin has been the drug of choice in the treatment of GRKP, but with the rise of carbapenem resistance, the colistin usage has been increased over the years leading to slow emergence of colistin resistance. Especially, if these antibiotics are not used in combinations, they are not enough as therapeutic agent’s leads to treatment failure and high mortality rates. The high rate of resistance to colistin and carbapenems is worrisome, but the MIC of colistin and carbapenems helps the clinician to choose the drug in appropriate combinations.

Shah et al. conducted a study in a tertiary care hospital in Mumbai, the purpose of this study was to evaluate the efficacy of colistin-carbapenem combination against Carbapenem-resistant GNB (CRGNB) infection in a clinical study and an in vitro synergy study using Best. Overall, 60.6% clinical success was observed in patients receiving colistin-carbapenem combination against CRGNB infection [20].

Tumbarello et al. [29] in his study emphasized the importance of combination therapy in carbapenemase-producing Klebsiella pneumonia. Combination therapy with tigecycline, colistin, and meropenem (MIC up to 8 ug/ml) was associated with lower mortality.

In another study by Daikos et al. [30] stated that combination therapy with carbapenem MIC of 8 was more beneficiary in severely ill patients by lowering the mortality rate.

CONCLUSION

The present study showed the emergence of colistin resistance among clinical isolates of K. pneumoniae which are alarming in ICUs. It is important to determine the MIC of colistin to formulate an effective treatment protocol, even for the resistant isolates. For the resistant cases, colistin should be used in combination with other antimicrobials for therapy. Necessary infection control precautions and increased awareness to prevent further rise in the drug resistance against this last resort of antimicrobials is important. A restricted and rational use of the colistin is the need of hour.

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