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Klebsiella pneumoniae Carbapenemase Detection Methods: Review article

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ABSTRACT

Background: The Klebsiella tribe is a subfamily of the Enterobacteriaceae family, which includes the Klebsiella genus. The organisms are named after the German microbiologist Edwin Klebs, who worked in the 19th century. Klebsiella are Gram-negative bacteria that are nonmotile and rod-shaped. Their polysaccharide capsule is easily recognizable. Carbapenems are a type of β-lactam antibiotic that are effective against a wide range of microorganisms, including both Gram-positive and Gram-negative bacteria, as well as aerobic and anaerobic strains.

Objective: Review of the literature on Klebsiella pneumoniae carbapenemase detection methods.

Methods: We looked for data on Klebsiella pneumoniae, detection methods in scholarly journals and databases including PubMed, Google Scholar, and Science Direct. Only, the most recent or extensive study published between May 2017 and May 2022 was taken into account. The writers also analyzed similar works cited in their work. Lack of resources to translate documents written in languages other than English has led to their neglect. It was generally recognized that scientific research did not include research that was not published in a peer-reviewed journal, presented orally, or presented as a conference abstract or dissertation.

Conclusion: Implementing effective antibiotic therapy depends on the prompt and accurate laboratory detection of carbapenemase-producing bacteria. Disk-diffusion testing or increased minimum inhibitory concentrations (MICs) for carbapenems are typically used for screening for carbapenemase synthesis. A rapid turnaround time for carbapenemase detection technologies is desirable for prompt regulation. As discussed in Enterobacteriaceae and Acinetobacter baumannii, this could be complicated by the fact that MICs to carbapenems may be raised yet still within the susceptible range or even low.

Keywords: Klebsiella pneumoniae, Detection methods.

INTRODUCTION

The Klebsiella tribe is a subfamily of the Enterobacteriaceae family, which includes the Klebsiella genus. The organisms are named after the German microbiologist Edwin Klebs who worked in the 19th century. Klebsiella are Gram-negative bacteria that are nonmotile and rod-shaped. Their polysaccharide capsule is easily recognizable. This capsule protects the entire cell, explains the organism's bloated gram stain appearance, and several host defenses **(1).**

Klebsiella can be found almost anywhere on Earth. It's possible for them to set up shop on human skin, in the throat, or in the gut. Colonization of sterile wounds and urine is also possible. The cost of transportation varies widely between studies. Many Klebsiella species are thought to be commensal in the biliary tract, and they have also been found in the colon and intestines. Endotracheal intubation, compromised host defenses, and antibiotic usage have all been linked to oropharyngeal carriage **(2).**

Most human infections are caused by two species within this genus: K pneumoniae and K oxytoca. These microbes are opportunistic pathogens that can be found in the wild and in the mucosal surfaces of mammals. Both patients' digestive systems and healthcare workers' hands are major sources of harmful reservoirs of infection. Nosocomial outbreaks occur when an organism spreads fast within a healthcare facility **(3).** In addition to a history of antibiotic usage, other factors that increase the probability of infection include being in poor health. These species' ability to acquire resistance to several antibiotics and to potentially transmit plasmids to other organisms has made their acquisition a major problem in hospitals worldwide **(2).**

K. pneumoniae is able to outwit the host's cellular and humoral defenses in addition to the physical and chemical barriers present during an infection. The vulnerable cell is then attacked by the host organism once it has entered the host, a process that is related to pattern recognition. The monocyte/macrophage system is involved in the innate vulnerable response after identification by the receptor activates the product of central vulnerable intercessors. In addition to its phagocytic abilities, this system controls the sensitive response to cytokines and chemokine products. When the body detects an illness, neutrophils rush in to begin fighting it. Important cytokine proteins at this stage include IL-8 and IL-23, both of which contribute to the transformation of a granulopoiesis response **(4).**

Preclinical research has shown that neutrophil myeloperoxidase and lipopolysaccharide-binding protein may play a part in the host's resistance against K pneumoniae infection. Elastase, an enzyme involved in the pathophysiology of several tissue-destructive disorders, is hypothesized to be oxidatively inactivated by neutrophil myeloperoxidase. A protein that binds lipopolysaccharides increases the movement of components of bacterial cell walls into inflammatory cells **(5).**

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Multiple mechanisms allow bacteria to survive the host's initial defenses. The key factor in their pathogenicity is the polysaccharide capsule they contain. The capsule is made up of acidic polysaccharides that are quite complicated. The bacterium is shielded from phagocytosis by polymorphonuclear granulocytes by this thick coating. The capsule also protects the bacteria from serum factors that would normally kill them. Inhibiting the activation or absorption of complement components, particularly C3b, is crucial to this end. Bacteria also secrete a variety of adhesins. Both fimbrial and nonfimbrial forms exist, with fimbrial forms having a narrower receptor specificity. The ability of microbes to cling to host cells is essential to the spread of infection, and these aid in this process [(6)].

Another component of bacteria's pathogenicity is lipopolysaccharides (LPS). By activating complement, they trigger the preferential deposition of C3b onto LPS molecules in locations far from the bacterial cell membrane. The production of the membrane assault complex (C5b-C9) is thereby blocked, protecting the membrane and keeping the bacteria alive [(7)].

Antibiotic-resistant bacteria often originate from K. pneumoniae. The rate of resistance in K. pneumoniae is clearly rising over time. There is a wide range of resistances from country to country. Multi-drug resistant (MDR) K. pneumoniae is endemic in Eastern and South-Western Europe and Mediterranean countries due to ESBL production, with more than 50% displaying non-susceptibility to third-generation cephalosporins, fluoroquinolones, and aminoglycosides. While, practically everywhere was devoid of carbapenem-resistant Klebsiella in 2005, non-susceptible rates of 40-60% have now appeared in numerous countries due to the spread of carbapenem-resistant K. pneumonia (CRKP) [(8)].

Laboratory detection:

Implementing effective antibiotic therapy depends on the prompt and accurate laboratory detection of carbapenemase-producing bacteria. Carbapenemase production is typically indicated in screening by smaller-than-usual inhibition zones around carbapenem discs in routine disk-diffusion testing or by higher MICs for carbapenems**.** Porin loss and activation of efflux pumps are two examples of additional resistance mechanisms that might increase a bacteria's resistance to carbapenems. Recommended cutoffs for carbapenem susceptibility have been developed by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST), although there is no consensus on them. However, there are still low-resistance strains that aren't being taken seriously. Epidemiologic cutoffs have been established by EUCAST to distinguish between wild-type and carbapenem-resistant isolates [(9)].

A rapid turnaround time for carbapenemase detection technologies is desirable for prompt regulation. This could provide a problem in finding carbapenemase manufacturers. Carbapenemase can have extremely low MICs, especially in species that produce OXA-48. In most cases, a smaller inhibition zone surrounding a disc of ertapenem is used in the screening process [(10)].

However, a uniform approach that includes the necessary laboratory tests has not yet been developed. Despite its limited sensitivity and specificity, for phenotypic detection of carbapenemase synthesis, CLSI recommends using the modified Hodge test. Inhibitor-based tests include those that use a carbapenem (like meropenem) or a cephalosporin (like ceftriaxone) in conjunction with an inhibitor (such EDTA or phenanthroline as MBL inhibitors, or phenylboronic acid as a KPC inhibitor) (like ceftazidime). The use of temocillin disc (or temocillin disc combined with avibactam) has been proven to be effective against class D carbapenemases, despite the lack of a specific inhibitor for these enzymes [(11)].

The Carba NP (short for "Northern-Poirel") test is a straightforward biochemical assay that uses the color change of an indicator caused by a reduction in pH to detect the hydrolysis of imipenem. Most microbiology labs can use it, However, spectrophotometric detection of carbapenem hydrolysis with or without an inhibitor remains the gold standard for detecting carbapenemase synthesis. A new carbapenemase detection approach has been described recently. Carbapenem inactivation method (CIM) was the name given to the test since it relied on the breakdown of meropenem by carbapenems [(9)].

Two hours are spent incubating a 10-ug meropenem disc with a carbapenem-resistant microbe suspension in water. The disc is taken out of the suspension after incubation and then placed on a Mueller-Hinton agar plate that has already been infected with the carbapenem-sensitive bacterium (usually Escherichia coli). Carbapenemase production by a carbapenem-resistant bacteria will cause hydrolysis of meropenem, allowing the indicator (carbapenem-susceptible) microbe to flourish near the disc. According to recent studies, the CIM approach is just as sensitive as the Carba NP test. Some carbapenemases in Enterobacteriaceae can be better detected using a modified CIM test, which entails preparing the bacterial suspension in tryptic soy broth and increasing the length of incubation to 4 hours [(10)].

Mass spectrometry (matrix-assisted laser desorption/ ionization-time of flight—MALDI-TOFF) has recently allowed for the quick identification of KPC carbapenemase (in 45 min) or MBL through the measurement of degradation of carbapenem-molecules (150 min). With the sensitivity and specificity issues of phenotypic testing eliminated, the identification of carbapenemase genes in the clinical laboratory might be greatly enhanced by the use of simplex or multiplex

PCR, real-time PCR, or hybridization assays. However, molecular techniques call for high-priced tools and expert laboratory personnel [11].

Nucleic acid-based techniques:

Molecular determinants of carbapenemase synthesis can be directly identified using nucleic acid-based detection methods such polymerase chain reaction (PCR), microarray, and whole genome sequencing [12].

Several factors should be considered before deciding on a carbapenemase detection test, such as the prevalence of carbapenemase in the area, the molecular epidemiology of the region, the diagnostic performance characteristics of the test, the amount of time and effort required to complete the test, the cost, and the turnaround time (TAT). Same-day results are ideal for the TAT, which is critical for therapeutic decision-making and infection management. Use, process, regulatory status, necessary equipment, and reagent preparation needs should all be taken into account, as should the organisms to be tested (such as Enterobacteriaceae and /or glucose-non fermenting Gram negatives). Unfortunately, there is no existing test that satisfies all of these requirements. There are many options available, so labs can pick the method that works best for them [13].

Treatment of carbapenem resistance klebsiella pneumoniae:

7% of 299 patients infected at admission and 27% acquired CR-KP during their stay in a medical-surgical ICU, according to a prospective surveillance study. Independent risk factors for acquiring CR-KP were recent surgery and sickness severity (SOFA score). 47% of patients who were colonized became infected. The characteristics that put a person at risk for colonization also put them at risk for CR-KP infection [14].

Patients who had a stem cell or solid organ transplant have also been linked by certain writers to an increased risk of CR-KP infection. Only one study has looked into what can put a colonized person at risk for CR-KP infection. The risk factors for developing CR-KP infection included a history of invasive procedures, diabetes mellitus, a solid tumor, a tracheostomy, a urinary catheter, and prior exposure to an antipseudomonal penicillin in 42 (9%) of 464 individuals who were found to be rectal carriers of CR-KP by stool cultures. Last but not least, a significant pool of endemic CR-KP resistance has been found among residents of long-term care, post-acute care, and nursing homes [15].

Principles of antimicrobial pharmacokinetics and pharmacodynamics one of three approaches is often used to treat CR-KP. First, you might try giving your patient a larger dose of a first-line antibiotic (such meropenem, fluoroquinolone, or aminoglycoside) to see if it helps. However, the minimum inhibitory concentrations (MICs) of some CR-KP isolates to first-line medicines are quite high, making it necessary to use extremely large doses with unacceptable toxicity to reach the pharmacokinetic and pharmacodynamic (PK/PD) exposures necessary for efficacy [16].

The alternative is to employ a Gram-negative antibiotic for which resistance has not yet emerged, known as a "second-line antibiotic" (e.g., colistin, tigecycline, gentamicin, Fosfomycin). The urine, blood, and lungs are common sites for the emergence of CR-KP, but many second-line medicines are either more toxic than first-line medications or have major PK inadequacies that limit their effectiveness there [17]. Even second-line antimicrobials pose a threat of rapid resistance development if used alone. Combining first- and second-line antibiotics is a last resort for treating CR-KP infections because of the hope that synergistic interactions between antibiotics will reduce the need for extremely high antibiotic doses, slow the development of resistance, and compensate for the PK weaknesses of individual agents. Given the lack of effective single-drug treatments for CR-KP, it is not unexpected that many doctors have turned to combination therapy [18].

However, proper dose of a single medicine is still required, even when using combination therapy. Numerous studies have shown that PD-optimized dosage regimens at higher doses are a crucial part of successful combination therapies for CR-KP infection. Drug kinetics and pharmacodynamics (PK/PD) concepts and dose considerations for the commonly used antibiotics in the treatment of CR-KP infections (meropenem, colistin, tigecycline, gentamicin, and Fosfomycin) discussed. Healthcare-associated infections in the critically ill and immunocompromised continue to be treated with antipseudomonal carbapenems (doripenem, IMP & meropenem) despite rising resistance to these antibiotics. Carbapenems are effective against a wide variety of infections because they diffuse widely throughout the body after intravenous infusion, including the epithelial lining fluid of the lung, the blood, the urine, and the central nervous system [15].

In order to treat infections with increased MICs, it is best to provide the drug at greater dosages via extended or continuous infusion because carbapenems are swiftly removed (unchanged) through the kidney with plasma half-lives ranging from 1 to 2 hours [18].

The use of carbapenems at larger doses and over longer periods of time raises a number of logistical challenges. For one, once reconstituted, these antibiotics are rather unstable at room temperature, necessitating frequent bag changes every 4-6 hours. Researchers have found that meropenem can be provided in a continuous-infusion fashion if infusion bags are kept at 23 °C for up to 8 hours [15].

In comparison with meropenem and doripenem, IMP is less stable at room temperature and has a higher risk of seizures at high dosages, hence it is rarely utilized for high-dose, prolonged-infusion therapy. Finally, some medications that are incompatible with carbapenems may have more difficult administration

schedules if they need to be given at different times than carbapenems. However, in the context of CR-KP treatment, the advantages of prolonged-infusion regimens usually outweigh the patient's practical difficulties [17].

Initial antibiotic selection:

Antibiotic resistance is widespread among Klebsiella species. It is believed that plasmids are responsible for this trait. Risk factors for contracting these strains include prolonged hospital stays and the use of invasive treatments [3].

The treatment required is contingent upon the affected organ. Initial treatment of patients suspected of having bacteremia is often an empirical process. The susceptibility patterns of bacteria in a given area should inform the selection of an antibiotic. Confirmation of bacteremia allows for adjustments to treatment [1].

When other treatment options are insufficient or unavailable, ceftazidime/avibactam is licensed for the treatment of severe urinary tract infections (UTIs), including kidney infections (pyelonephritis). Ceftolozane/tazobactam and ceftazidime/avibactam are two of the newer beta-lactam/beta-lactamase combination antibiotics available. Ceftazidime/avibactam is effective against carbapenem-resistant Enterobacteriaceae such K. pneumoniae, but not vice versa [2].

Meropenem/vaborbactam (Vabomere) is a new carbapenem/beta-lactamase inhibitor that targets carbapenem-resistant Enterobacteriaceae (CRE) such E. coli and Klebsiella pneumoniae by preventing the development of enzymes that block carbapenem antibiotics. For treatment of CRE-related cUTI, the Food and Drug Administration (FDA) approved meropenem/vaborbactam in August 2017 [4].

If a patient has an allergy to beta-lactam antibiotics, aztreonam may be an option. Patients with a severe beta-lactam allergy or a carbapenem allergy can still be effectively treated for susceptible isolates by switching to a quinolone. Ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanate, ceftazidime, cefepime, levo-floxacin, norfloxacin, moxifloxacin, meropenem, and ertapenem are some of the other antibiotic combinations utilized to treat resistant isolates. Klebsiella pneumonia treatment outcomes vary widely. It is clinically sound to start patients with severe infections on a short course (48-72 h) of combination therapy with an aminoglycoside and then transition to an extended-spectrum cephalosporin once susceptibility has been demonstrated [19].

Antibiotic considerations for resistant infections:

Resistance to ampicillin, amoxicillin, and ticarcillin can be provided by beta-lactamases, which are constitutively expressed and typically produced at low levels. The plasmid-mediated, multidrug-resistant (TEM or SHV kinds), and detectable by in vitro resistance to ceftazidime and aztreonam ESBLs are a major public health concern. In the Escherichia coli ST131 lineage, the prevalence and proliferation of CTX-M type ESBLs have been observed. CTX-M ESBLs hydrolyze ceftazidime substantially less than other third- and fourth-generation cephalosporins [2].

Antibiotics such as carbapenems, penicillins, cephalosporins, fluoroquinolones, and aminoglycosides are ineffective against carbapenemase-producing isolates. Colistin (the drug of choice for urinary tract infections), tigecycline, and occasionally intravenous fosfomycin are the only alternatives for treatment [1].

Many CR-KP isolates, including those resistant to tigecycline and colistin, are susceptible to fosfomycin because it is a broad-spectrum, time-dependent bactericidal antibiotic that inhibits an early enzymatic step in bacterial cell wall formation. Among the pharmacokinetic (PK) properties that make fosfomycin an attractive drug for treating CR-KP infections in critically-ill patients are its high concentrations in the urine, plasma, lung, cerebrospinal fluid, and muscle and its minimal risk for nephrotoxicity. Recent research analyzed the side effects of 72 different courses of fosfomycin therapy and found that the most common were hypokalemia (26% of patients), injection-site pain (4%), and heart failure (3%), all likely due to the high salt concentration of the intravenous formulation. Especially when dealing with K. pneumonia, resistance to fosfomycin is likely to emerge quickly if the antibiotic is used alone as treatment [20].

Patients with bacteremia may have a better chance of survival if they receive treatment that combines colistin, tigecycline, and carbapenem. Think about how well the medicine can go into the lung tissue to treat pneumonia, or how concentrated the urine is to treat urinary tract infections. Percutaneous drainage is a possible treatment option for liver abscess [4].

Community-acquired pneumonia

Without therapy, the death rate could be as high as 50%. Empirical coverage for gram-negative pathogens, intensive ventilation, and supportive care are the cornerstones of treatment for this extremely rare illness. In addition, pulmonary gangrene, lung abscess, and empyema can all be detected through clinical and radiologic surveillance. Coverage for K pneumoniae infection acquired in the community is provided by third-generation cephalosporins or quinolones. One study found that using aminoglycosides in combination was more effective than using them alone, although this benefit was not seen in subsequent investigations. Against K pneumoniae, macrolides are completely ineffective. At least 14 days of antibiotic treatment is required [19].

Nosocomial K pneumoniae pneumonia

Pick antibiotics that work well on their own. Imipenem, third-generation cephalosporins, quinolones, or aminoglycosides may be administered singly or in combination as part of an antibiotic treatment plan.

Verifying susceptibility is essential. A minimum of 14 days of treatment is recommended. A chest tomography scan may be helpful in ruling out entities that can be treated with debridement or drainage if response is sluggish. Patients who show a rapid response to intravenous treatment can safely transition to oral quinolones if the isolate is responsive [(2)].

K pneumoniae UTI

Most oral medicines, with the exception of ampicillin, can be used to treat simple cases caused by susceptible strains. Treatment for only three days with a monotherapy that works is all that is needed. Oral quinolones, intravenous aminoglycosides, imipenem, aztreonam, third-generation cephalosporins, and piperacillin/tazobactam are also options for treating complicated infections. The average course of treatment lasts between 14 and 21 days. Fever is treated with intravenous medications until they work. Removal of a urinary catheter or surgical repair of an anatomical defect are two other options [(4)].

Other K pneumoniae infections

Cholangitis is typically treated empirically with a combination of a beta-lactam antibiotic and an aminoglycoside. There is a lack of comparable evidence to determine whether or not this treatment is superior. When treating acute suppurative cholangitis, ciprofloxacin alone is just as effective as a combined treatment. At least 10 days of treatment with antibiotics is required. Possible need for biliary decompression [(3)].

Adults almost never get meningitis caused by Klebsiella. Shunts in children can be complicated by nosocomial infections. Superior central nervous system penetration makes third-generation cephalosporins the medications of choice. Cefotaxime has been shown to be effective, and meropenem is a good backup option. Shunt removal due to infection is one supplementary treatment option. Higher relapse rates have been observed in patients treated with shorter durations of medication, thus a minimum of three weeks of treatment is recommended [(1)].

Endophthalmitis and endocarditis due to Klebsiella are extremely unusual. Endophthalmitis treatment can be administered intravenously, intravitreally, or both. While intravenous ceftazidime and aminoglycosides have the most clinical experience, they also produce the lowest medication concentrations at the infection site when used alone. Intravenous aminoglycoside and beta-lactam antibiotic therapy has been used to treat endocarditis. There is a lack of evidence to determine how long antibiotic treatment should last, however 6 weeks is typically recommended [(19)].

CONCLUSION

Implementing effective antibiotic therapy depends on the prompt and accurate laboratory detection of carbapenemase-producing bacteria.

Disk-diffusion testing or increased minimum inhibitory concentrations (MICs) for carbapenems are typically used for screening for carbapenemase synthesis. A rapid turnaround time for carbapenemase detection technologies is desirable for prompt regulation. As discussed in Enterobacteriaceae and Acinetobacter baumannii, this could be complicated by the fact that MICs to carbapenems may be raised yet still within the susceptible range or even low.

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