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Molecular Detection of *Maga* Gene Among Clinical Isolates of *Klebsiella Penumoniae*

Summera Rafiq	Dept of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai-18
D. Priyadharshini	Dept of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai-18
SK. Jasmine Shahina	Dept of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai-18

ABSTRACT

Klebsiella infections are caused mainly by *K.pneumoniae* and *K.oxytoca*. They are opportunistic bacterial pathogens. associated with nosocomial infections such as urinary. tract infection (UTI), pneumonia and septicaemia. A new virulence gene which is mucoviscosity-associated gene A *(magA)*, has recently been identified in this pathogen. The present study was taken up to screen *Klebsiella pneumoniae* isolated from different clinical samples for the presence of *magA* gene. A total of 26 *K.pneumoniae* were isolated from pus swabs 2 (40%), 14 (70%) from urine samples and 10 (67%) from stool samples. A total of 8/26 (31%) isolates of *K.pneumoniae* showed the presence of *magA* gene. They showed high resistance towards imipenem (88%) followed by cefepime (67%), gentamycin (54%), nalidixic acid (50%), amikacin (42%), norfloxacin (42%) and ciprofloxacin (25%). They showed high sensitivity towards ciprofloxacin (75%) followed by amikacin (58%), norfloxacin (58%), nalidixic acid (50%), gentamycin (45%), cefepime (33%) and imipenem (12%).

KEYWORDS Klebsiella, magA gene, hyperviscosity, antibiogram.

Introduction

K.pneumoniae is an opportunistic and major hospital-acquired pathogen, causing urinary tract infections, nosocomial pneumonia, bacteraemia and septicemia (Ko, Paterson et al., 2002). For the first time in 1998, a new type of invasive *K.pneumoniae* emerged in Taiwan, which was typically presented as a community-acquired primary liver abscess (1, 2, 3). Several reports, especially from the Asia Pacific region and the United States, have also shown that this pathogen has become the predominant cause of liver abscess (4,5). A new virulence gene which is mucoviscosity-associated gene A *(magA),* has recently been identified in this pathogen. magA is detected in a vast majority of *K.pneumoniae* liver abscess isolates and is associated with hypermucoviscosity (HV) and resistance to killing by human serum and phagocytosis.

The regulator of the mucoid phenotype *(rmpA),* a gene known as an extracapsular polysaccharide synthesis regulator, can positively control the mucoid phenotype of *K. pneumoniae*. This mucoid phenotype is distinct from capsule production and results from overproduction of extracellular polysaccharide, which is encoded by the chromosome but is positively controlled by *rmpA* located on a plasmid. Although not directly shown by a positive string test result, we believed that the glistening mucoid and viscid consistency of the colonies conferred by *rmpA*(6) was similar to the so-called "hypermucoviscosity phenotype" described by Fang et al., (2004) (7).

Materials and Methods

Klebsiella pneumoniae were isolated from different clinical specimens like pus, urine, stool and wounds. The wound samples were collected with the help of sterile cotton swabs and the stool and urine samples were collected in sterile containers under aseptic conditions by standard procedures and were processed according to standard guidelines. Samples were inoculated on to blood agar, Mac Conkey agar, and nutrient agar for primary isolation. Based on the colony morphology and gram staining the gram-negative rods were further identified based on the biochemical methods as per standard protocols.

Antibiotic sensitivity testing

Antibiotic sensitivity testing was carried out by Kirby Bauer disc diffusion method (8) for the following antibiotics- (in µg/disc) - ciprofloxacin (30mcg), imipenem (30mcg), cefepime (30mcg), amikacin (30mcg), gentamycin (30mcg), norfloxacin (5mcg), nalidixic acid (30 mcg).

Molecular detection of *mag A* gene

Amplification of *magA* (198 bp) gene was done in 25 micro/l reaction with 10X standard PCR buffer {100mM Tris-HCL pH 8.3, 500Mm kcl;1.5mM MgCl2} (NEB), 200mM dNTP mix (sigma), 25pmol of each primer (sigma), 2.5 U of Taq DNA polymerase (NEB) and 1 micro litre template DNA. The following were the primers used in the study-(Table 1)

Table 1. Primer sequences

Target Gene	Oligonucleotide (primer) Sequence	Product Size	Reference
magA Forward	5'- GCCAACAATTCCCGTTTCTGCTGC-3'	198bp	Zamani.A et al., 2013(9)
magA Reverse	5'- ACGGAGCAATATGGCCAGTCCG-3'		

PCR was performed with the following cycling conditions- initial denaturation at 94°C for 5 minutes, denaturation-94°C for 1 minute, annealing-58°C for 1 minute, extension-72°C for 1 minute and final extension-72°C for 5 minute.

Results

In the present study 26 isolates of *Klebsiella pneumoniae* were obtained from various clinical specimens like pus, urine, and stool. The following was the prevalence of *Klebsiella pneumoniae* from various samples collected. 2(40%) from the pus

swabs, 14(70%) from the urine samples and 10 (67%) from the stool samples (Table 2).

Table 2. Prevalence of K. Pneumoniae from different samples

S.No.	Source of sample	Total number of samples collected	Percentage of *Klebsiella pneumoniae* from different samples
1	Pus	5	2(40%)
2	Urine	20	14(70%)
3	Stool Samples	15	10 (67%)

Antibiotic sensitivity test

Isolates of *Klebsiella pneumoniae* showed high resistance towards imipenem (88%) followed by cefepime (67%), gentamycin (54%), nalidixic acid (50%), amikacin (42%), norfloxacin (42%) and ciprofloxacin (25%). They showed high sensitivity towards ciprofloxacin (75%) followed by amikacin (58%), norfloxacin (58%), nalidixic acid (50%), gentamycin (45%), cefepime (33%) and imipenem (12%).

Detection of *magA* gene among clinical isolates of *Klebsiella pneumoniae*

A total of 8/26 (31%) isolates of *Klebsiella pneumoniae* showed the presence of *magA* gene, while 18/26 (69%) of the isolates were found to be negative for *magA* gene. (Chart 1, Figure 1)

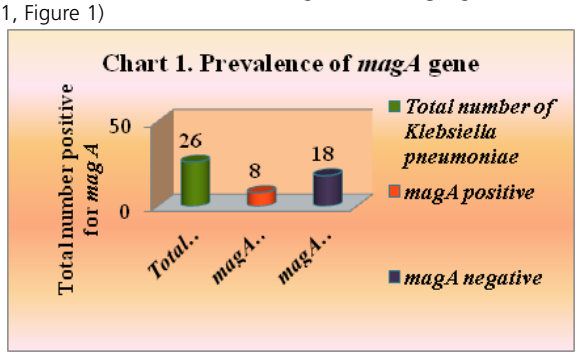
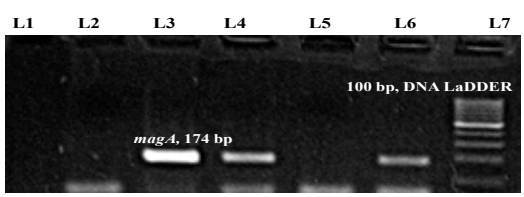


Figure 1

Gel picture showing *magA* gene



L1, L2 and L5- Negative, L3, L5 and L6- *magA* positive, L7- 100

Discussion

Among the human pathogenic bacteria Klebsiella species are very notable. In recent years Klebsiella have become important pathogens in noscomial infections. Epidemic and endemic noscomial infections caused by Klebsiella species are leading causes of morbidity and mortality (10). In addition to being the primary cause of respiratory tract infections like pneumonia, rhinoscleroma, ozaena, sinusitis and otitis.

In the present study, the following was the prevalence of *Klebsiella pneumoniae* from various samples collected. 2(40%) from the pus swabs, 14(70%) from the urine samples and 10 (67%) from the stool samples. In the present study culture positivity for Klebsiella from pus samples was 70%, which is similar to Valarmathi et al., (2013)(11) and 67% in urine samples which is in agreement with Sarathbabu et al., (2012) (12).

Knowledge about the common organisms associated with infections, the resistance patterns of these bacterial strains in a geographical area will help to guide appropriate and judicious antibiotic use, formulate antibiotic policies and for infection control intervention programmes. Resistance to ciprofloxacin was high in Klebsiella, it was 62.2% in *K.pneumoniae* and 45% in *K.oxytoca* by a study done by Aktas et al., (2002) (13). Our study reported a lesser resistance towards ciprofloxacin which was 25%. Aminoglycosides have good activity against clinically important gram negative bacilli. Amikacin showed good activity with 58% *K.pneumoniae* isolates. This is lower than the study conducted in Doha Qatar by Ahmed et al., (2013) (14).

In the present study, a total of 8/26 (31%) isolates of *Klebsiella pneumoniae* showed the presence of *magA* gene, while 18/26 (69%) of the isolates were found to be negative for *magA* gene. In Fang's study, 52 out of 53 (98 %) *K.pneumoniae* isolated from liver abscess carried this specific virulence gene. Fang believed that *magA* gene is exclusively limited to liver abscess and HV positive phenotype (15). With extension of global research in other countries such as North American countries, Singapore and Korea, on Klebsiella isolates, they showed *magA* gene isolated from other cases like acquired bacteraemia, sepsis, meningitis and endophthalmitis (16). In contrast to Fang's studies, these samples included HV+ and HV-phenotypes. Therefore, based on the results of the present as well as other studies, containing HV+ phenotype is not a certain reason for the presence of *magA* gene since the HV- phenotype may have *magA* gene, too (Struve et al., 2005) (17).

Conclusion

In the present study 26 isolates of *Klebsiella pneumoniae* were obtained from various clinical specimens like pus, urine, and stool. A total of 8/26 (31%) isolates of *Klebsiella pneumoniae* showed the presence of m*agA* gene, while 18/26 (69%) of the isolates were found to be negative for *magA* gene In addition, the presence of HV+ phenotype was not associated with *magA* gene. Most of the isolates had a high level of resistance to antibiotics. Antibiotic susceptibility testing should be carried out to help in the choice of systemic drugs. Continuous monitoring of antimicrobial susceptibility pattern in individual settings together with their judicious use is emphasized to minimize emergence of drug resistant bacteria.

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