Molecular Detection of Maga Gene Among Clinical Isolates of Klebsiella Pneumoniae

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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 septicemia. 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 opportunity 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 associated with nosocomial infections such as urinary tract infection (UTI), pneumonia and septicemia. 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%).

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 microl reaction with 10X standard PCR buffer (100mM Tris-HCL pH 8.3, 5000Mm 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

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Oligonucleotide (primer) Sequence</th>
<th>Product Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>magA Forward</td>
<td>S’- GCCAACAAATTCCCGTTTGTGTCG-3’</td>
<td>198bp</td>
<td>Zamani A et al., 2013(9)</td>
</tr>
<tr>
<td>magA Reverse</td>
<td>S’- ACGGAGCAATATGGCCAGTCCG-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Source of sample</th>
<th>Total number of samples collected</th>
<th>Percentage of Klebsiella pneumoniae from different samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pu  s</td>
<td>5</td>
<td>2(40%)</td>
</tr>
<tr>
<td>2</td>
<td>Urine</td>
<td>20</td>
<td>14(70%)</td>
</tr>
<tr>
<td>3</td>
<td>Stool Samples</td>
<td>15</td>
<td>10 (67%)</td>
</tr>
</tbody>
</table>

Antibiotic sensitivity test
Isolates of Klebsiella pneumoniae showed high resistance towards imipenem (88%) followed by cefepime (67%), gentamicin (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

Discussion
Among the human pathogenic bacteria Klebsiella species are very notable. In recent years Klebsiella have become important pathogens in nosocomial infections. Epidemic and endemic nosocomial 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, rhinoceromal, 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. In the present study culture positivity from the pus samples was 70%, which is similar to Valarmathi et al., (2013)(11) and 67% in urine samples.

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 highest 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 magA 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.
REFERENCES


