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Isolation and identification of *klebsiella pneumonia* causal-agent of pneumoniae from urine of childrens in hospitals of Quetta city

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Abstract

The pneumoniae is worldwide leading factor which cause mortality in children however, in under developing countries the burden of *klebsiella pneumoniae* is greater in under 5 year age children. Therefore proper and continuously surveillance and appropriate screening tests is necessary for the detection. The samples were collected from children up to 5 years of age affected with pneumoniae and showing main symptoms of pneumoniae. The colonies with morphology suggestive of *klebsiella pneumoniae* were further confirmed by mac Conkey agar, eosine methylene blue agar, gram staining, different biochemical tests and PCR. Urine samples were analyzed by different techniques in which about 24% samples were positive with *klebsiella pneumoniae* and 76% were negative. Where sex wise ratio among positive cases has been 11% cases were from female and 13% samples were positive from males. Where age wise distributed as 4% up to six months of age, 5% from six months upto one year, 7% from one year to two year, 6% in three years, 1% in four year and 1% in five year of age. Mostly all used drugs were affective against *klebsiella pneumoniae* but Sulfhamethoxazole showed (25mm) and Trimethoprim showed (22mm) zone of inhibition whereas some drugs found to be resistant against *klebsiella pneumoniae* which were Amoxicillin, Vancomycine, Lincomycin, Penicillin, Bacitaricin, Metronedazole and Erythromycin. While confirmation through PCR samples shown clear bands of 176bp of rcs A gene. The parents should have awareness about pneumoniae to protect children from such infectious disease.

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Introduction

The *Klebsiella pneumonia* is a bacterium with characteristics of gram negative have no motility (Podschun and Ullmann, 1998) found everywhere in environment. (Bagley, Brisse S *et al*., 1985,2006). Generally *Klebsiella pneumonia* is associated with high morbidity and mortality (Vernet *et al*., 1995). *Klebsiella pneumonia* isa major cause of morbidity and mortality in immune compromised patients. (Ariffin, 1997). This bacteria is responsible for nosocomial outbreaks due to its ability to spread rapidly and maintain their life cycle in the hospital environment, (Liu *et al*., 1998; Chetoui *et al*.). It can easily maintain its life in hospitals, multiply on environmental surfaces and make colony in the human bowels, skin bladder and respiratory tract (Struve, 2004. Macrae, *et al*., 2001). *Klebsiella pneumoniae* is an opportunistic pathogen that accounts for a significant proportion of hospital-acquired urinary tract infections, septicemia, soft tissue infections and pneumonia. (Podschun and Ullmann, 1998). The pneumoniae is worldwide leading factor which cause mortality in children. however, in under developing countries the burden of *klebsiella pneumoniae* is greater in under 5year age children. (W B R Johnson and A Abdulkarim, 2013) In new born period childrens has the greatest possibility and chances of deaths from pneumonia. In a field trial of community based management. In India more than 50% of child mortality happened due to pneumonia in new born childrens. (Bang *et al*., 1993). Whilein neonatal intensive care units (NICUs) *Klebsiella pneumoniae* has been recognized as one of the most persistent and repeated causes of outbreaks. (Skogberg *et al*., 1995-2002). 3.9 million of the 10.8 million deaths occur per year worldwide in neonatal life due to pneumoniae. in developing countries more than 96% neonatal deaths occur due to pneumoniae, . (Black *et al*., 2003:Barnett *et al*., 2001).

The annual incidence of pneumonia in Europe and North America in childrens which are younger than 5 years of age have 34 to 40 cases per 1000 which are higher than at any other time of life, except perhaps in adults older than 75 or 80 years of age. (Foy *et al*., 1979: Jokinen *et al*., 1993).

In the developing world, pneumonia is not only more common than it is in Europe and North America (Riley *et al*., 1983: Selwyn *et al*., 1990). In a child mortality, neonatal deaths account for over a third of the global burden (Lawn. *et al*., 2004). In many countries which are under developed conditions the infant death rates (deaths in the first 28 days of life) are as high as 40–50 per 1000 live births (Hyder *et al*., 2003).

The pneumoniae is worldwide leading factor which cause mortality in children. however, in under developing countries the burden of *klebsiella pneumoniae* is greater in under 5 year age children. *Klebsiella pneumoniae* is a serious threat to the children so therefore continuously surviellience and appropriate screening tests for laboratory detection is necessary.

Material and method

Collection of samples

The study was conducted in all major government hospitals of Quetta city and the samples were collected from children up to 5 years of age affected with pneumoniae and showing main symptoms of pneumoniae. Samples were collected in sterilized container and for neonatal patient's sterilized plastic urine bags were used for collection of urine for examination. These collected samples were quickly brought to the laboratory under feasible condition for microbiological analysis.

Isolation and identification

The samples were streaked on mac Conkey agar and kept in facultative jar at 37°C for 24 hours. The colonies with morphology suggestive of *klebsiella pneumoniae* were further confirmed by Eosine methylene blue agar, gram staining and different biochemical tests (catalase, oxidase, indole, methle red, vogusproskeur, urease test, citrate test. gelatin liquefaction test, sugars fermentations test).

Antibiotic disc sensitivity test

Antibiotic sensitivity test was performed by using disc diffusion Bauer technique and McFarland Turbidity Standard method 0.5 following CLSI protocols.

This test was done by using Mueller Hinton agar. The organism is accepted as sensitive and resistant by measuring the zones of inhibitions**.**

Pcr based detection of klebsiella pneumoniae

A 25µl reaction volume was used for all PCRs, with mixtures that consists the following ingredients: 12.5µl of PCR Master Mix reagents, 9.5µl of grade water, 1µM KP-27F3 and KP-27B3 primers, and the same amount of DNA template was used. The PCR cycling parameters were: initial PCR activation, 95°C for5min; amplification, 30 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s; final extension, 72°C for 10min. The products were separated with 1% agarose gel electrophoresis and stained with ethidium bromide and Images were documented.

Result

Total 100 urine samples were collected in which 24% were *klebsiella pneumoniae* positive and 76% were *klebsiella pneumoniae* negative as shown in (Fig. 1). It was monitored that among positive samples, 11% samples were from sandeman hospital Quetta 7% samples were from children hospital Quetta and 6% samples were from bolan medical complex Quetta as shown in (Fig. 2).

A predominance of male infant is apparent in almost all studies of pneumoniae in newborns. While in our study sex wise ratio showed that males were (13%) more affected as compared to female (11%) as shown in Fig. 3.

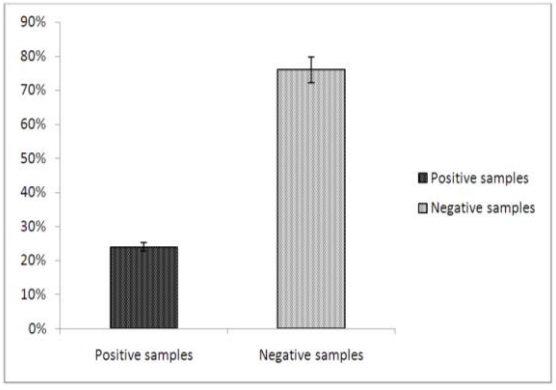

Fig. 1. Percentage wise distribution of positive and negative patients of *klebsiella pneumonia*.

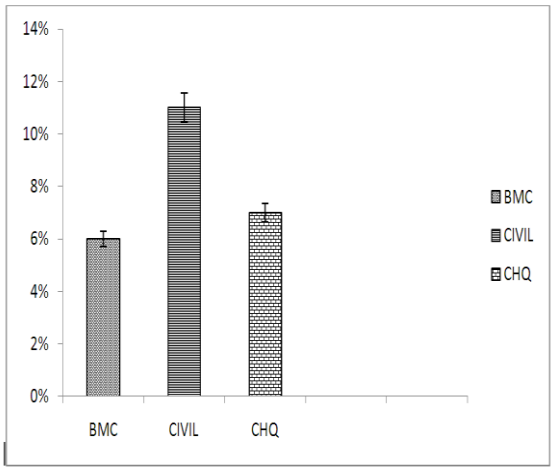

Fig. 2. *Klebsiella pneumoniae* positive patients in different hospitals.

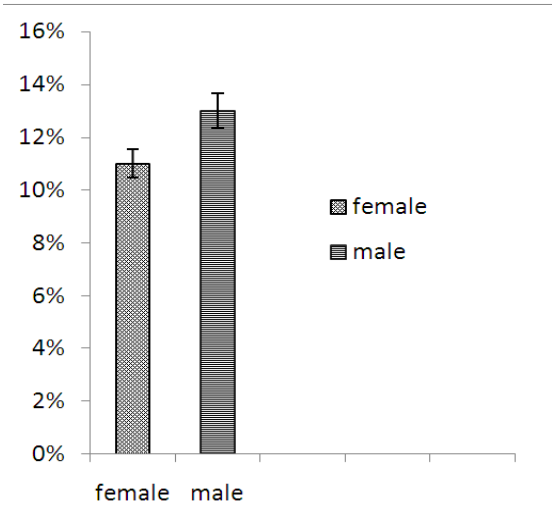

Fig. 3. Sex wise distribution of *klebsiella pneumoniae* in patients.

Microbial infection leading to pneumoniae is a major contributor to neonatal deaths in developing world the overall fatality rate due to pneumoniae in developing countries is estimated to be about 25%. Whereas age wise distribution showed 9% in one year, 7% in two years, 6% in three years, 1% in four years and 1% in five years of age as shown in (Fig.-4).

Initial identification of bacterial isolates was performed by all biochemical tests. Klebsiella pneumoniae was identified through differential medium, gram staining and different biochemical tests that are shown below in (Table 1).

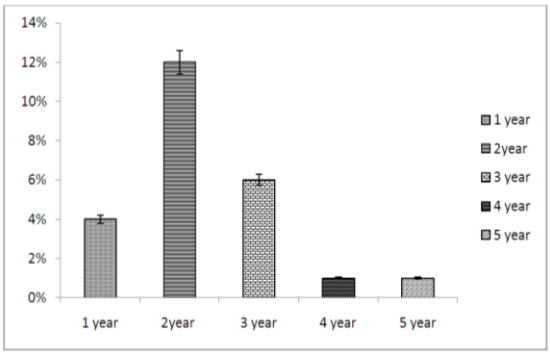

Fig. 4. Age wise distribution of *klebsiella pneumoniae* in patients.

Antibiotic disc sensitivity test

Antibiotics result showed that *Klebsiella* species were sensitive to Carbenicillin (8mm), Colistinsulphate (10mm), Kanamycin (15mm), Gentamycine (16mm), Ciprofloxacin (18mm), Tetracyclines (19mm), Trimethoprim (22mm) and Sulfamethoxazo (25mm). while resistant to Vancomycin Lincomycin penicillin bacitaricinDD2 Erythromycin Amoxicillin and Metronidazole. The zone of inhibitions of organisms against drugs are given in (Table 2).

Table 2. Antibiotic resistance and sensitivity test against *klebsiella pneumoniae*.

Class		Antibiotics	*Klebsiella pneumoniae*
1	Macrolides	Erythromycin	Resistant
		Penicillin G	Resistant
		Amoxicillin	Resistant
2	Penicillin	Carbencillin	Resistant
3	Polypeptide	Bacitaricin DD2	Resistant
4	Sulfonamides	Sulfhamethoxazole	25mm
5	Tetracycline	Tetracycline	19mm
		Gentamycine	16mm
		Kanamycine	15mm
6	Aminoglycoside	Streptomycin	9mm
7	Polypeptide	Colistin.sulphate	10mm
8	Quinolones	Ciprofloxacin	18mm
9	Glycopeptides	Vancomycine	Resistant
10	Lincosamides	Lincomycin	Resistant
		Metronedazole	Resistant
11	Others	Trimethoprim	22mm

Table 1. Different biochemical tests and sugar fermentation tests for identification *klebsiella pneumoniae*.

Biochemical test properties		*Klebsiella Pneumoniae*
Gram staining		-
Shape		Rod
Motility		-
Citrate test		+
Indole test		-
Methyl red test		-
Voges- Proskauer		+
Sugar fermentation tests	Glucose	+
	Sucrose	+
	Lactose	+
	Sorbitol	+
	Mannitol	+
	Trehlose	+
	dulicetol	
Catalase test		+
Urea hydrolysis test		+
Gelatin hydrolysis test		-
Casein hydrolysis test		-
Ornithine decarboxylase		-
Lysine decarboxylase		+

Confirmation of organism through PCR

Primers KP-27F3 with the sequence of (5' GGATATCTGACCAGTCGG 3') and KP-27B3 (5' GGGTTTTGCGTAATGATCTG 3') were designed to allow PCR amplification of 176 bp fragment of rcs A gene. The PCR amplification was positive for our isolation as shown in Fig. 5.

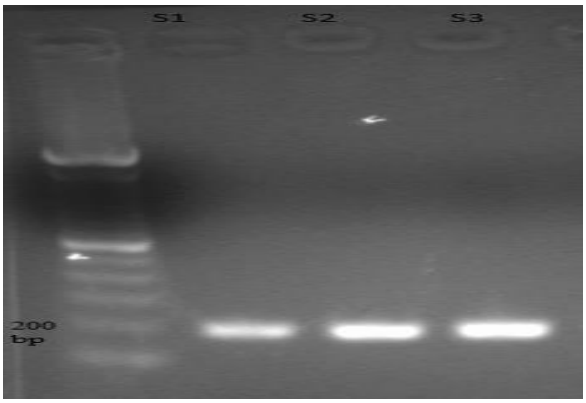

Fig. 5. PCR based identification of *klebsiella pneumoniae*.

Discussion

The pneumoniae is worldwide leading factor which cause mortality in children. however, in under developing countries the burden of *klebsiella pneumoniae* is greater in under 5 year age children. In our study total 100 urine samples were collected in which 24% were *klebsiella pneumoniae* positive and 76% were negative. It was monitored that among positive samples, 11% samples were from sandeman hospital Quetta, 7% samples were from children hospital Quetta and 6% samples were collected from bolan medical complex Quetta.

A predominance of male infant is appearant in almost all studies of pneumoniae in newborns (Cardero *et al*., (2004). While in our study sex wise ratio showed that males were (13%) more affected as compared to female (11%). Microbial infection leading to pneumoniae is a major contributor to neonatal deaths in developing world the overall fatality rate due to pneumoniae in developing countries is estimated to be about 25% (Qazi and Stoll, 2009). Whereas age wise distribution showed 9% in one year, 7% in two years, 6% in three years, 1% in four years and 1% in five years of age.

Initial identification of bacterial isolates was performed by all biochemical tests as described by Patricia *et al*., (1997). In our study *Klebsiella pneumoniae* was identified through differential medium, gram staining and different biochemical tests. Globally increasing resistance trends to multiple antibiotics in *K. pneumoniae* have complicated the management of these infections Erum *et al*., (2010). Antibiotics result showed that *Klebsiella pneumonia* were sensitive to Carbencillin (8mm), Colistin sulphate (10mm), Kanamycine (15mm), Gentamycine (16mm), Ciprofloxacin (18mm), Tetracycline (19mm), Trimethoprim (22mm) and Sulfhamethoxazole (25mm). While resistant to Vancomycine, Lincomycin, Penicillin, Bacitaricin DD2, Erythromycin, Amoxicillin and Metronedazole.

Primers KP-27F3 with the sequence of (5' GGATATCTGA CCAGTCGG 3') and KP-27B3 (5' GGGTTTTGCG TAATGATCTG 3') were designed to allow PCR amplification of 176 bp fragment of rcs A gene as described by Derong *et al.,* (2015). The PCR amplification was positive for our isolation.

Conclusion

It was observed that the guardians and the parents of the children's belonged to backward area and have lack of awareness about look after of children's specially neonates. For the control of pneumoniae in children's Special look after is necessary in winter season and cold weather from the month of October to the March.

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