Indian Journal of Animal Sciences **85** (2): 126–128, February 2015/Article

Preparation of autogenous vaccine against multi drug resistant *Klebsiella pneumoniae* outbreak in a rabbit farm

P SUMITHA[1], K SUKUMAR [2] and M ARTHANARI ESWARAN[3]

Veterinary College and Research Institute, Namakkal, Tamil Nadu 637 002 India

Received: 11 October 2013; Accepted: 8 October 2014

ABSTRACT

Ailing and dead rabbits, brought to the Department of Veterinary Microbiology, VCRI, Namakkal, with a history of anorexia, severe respiratory distress, mucus discharge from the nostril, mild enteritis and recumbency few hours before death, were used for the study. Relevant samples were collected and subjected to cultural examination on nutrient and MacConkey media. *Klebsiella pneumoniae* was isolated in pure culture and identified by morphological, staining, cultural and biochemical characters. Antibiotic sensitivity test showed multi-drug resistance. Inactivated vaccine was prepared by mixing mineral oil, surfactants and *Klebsiella pneumoniae* culture in the ratio of 6:1:3. Sterility and safety checking tests were carried out and the vaccine was administered to the rabbits. It produced noticeable protection against the disease.

Key words: Inactivated vaccine, *Klebsiella pneumonia*, Rabbits, Resistance

Klebsiella pneumoniae is a facultatively anaerobic gram negative, rod to coccobacillus shaped, non motile and capsulated bacterium and is responsible for a variety of diseases in humans and animals. Although it is a member of the family *Enterobacteriaceae* and occurs as a minority commensal in the intestine, rarely cause infection in intestine. Rather it is an opportunistic invader of reproductive tract of mares, bovine mammary gland, and urinary tract of bitches. In domestic animals, it is recovered from pneumonia and suppurative infections of foals; cervicitis and metritis in mare; mastitis in cow; wound infections; urinary tract infections, enteritis, septicemia and pneumonia in dogs (Diane *et al*. 2000). *Klebsiella pneumoniae* infection in rabbit is rare and usually associated with enteric form of infection. *Klebsiella* organisms are often resistant to multiple antibiotics. The autogenous vaccine can be used to treat infections even if the causative bacterium is resistant to antimicrobial drugs. This communication deals with the multidrug resistant *Klebsiella pneumoniae* infection associated with respiratory form along with septicemia in rabbits and its control using autogenous vaccine.

MATERIALS AND METHODS

A rabbit farmer from Coimbatore district of Tamil Nadu, brought ailing and dead rabbits to the Department of Veterinary Microbiology, VCRI, Namakkal. History of anorexia, severe respiratory distress, mucus discharge from the nostril, mild enteritis and recumbency few hours before death in the affected rabbits and 20 % mortality was noticed in 1,200 rabbits. Clinical signs mostly exhibited in recently weaned animals.

Necropsy was conducted immediately after death of the rabbits. Relevant samples were collected and subjected to cultural examination on nutrient and MacConkey media according to Quinn *et al.* (1994). The isolate was identified based on the following preliminary tests such as colony morphology, appearance on gram stain, negative stain, motility, indole, catalase and oxidase test. The analytical profile index (API) 20 E strips was used for species level identification as per the manufacturer's instructions. Antibiotic susceptibility tests were done against 8 commonly used antibiotics such as enrofloxacin (10µg), ciprofloxacin (30µg), gentamicin (10µg), oxytetracycin (30µg), streptomycin (25µg), amoxicillin (30µg), amikacin (30µg) and cotrimoxazole (25µg), using the standard disk diffusion method on Mueller-Hinton agar, and the inhibition zone sizes were interpreted as recommended by the Clinical and Laboratory Standards Institute (2008).

Preparation of autogenous vaccine: Pure colonies of *Klebsiella pneumonia* isolate were inoculated into 10 ml of nutrient broth and incubated at 37°C for 4–6 h and then the culture was inoculated into 1 litre of nutrient broth and incubated for 24 h. Purity of the broth culture was checked by gram staining and inoculating into MacConkey agar and

Present address: [1]Graduate Assistant (sumithabvsc @gmail.com), [3]Assistant Professor (drarthu@gmail.com), Department of Veterinary Microbiology. [2]Professor and Head, (drksugu@gmail.com), Department of Epidemiology and Public Health, Tirunelveli.

it was inactivated with 0.5% formalin for overnight at 4p C. Sterility of the inactivated broth culture was checked by inoculating into MacConkey agar plates. After the sterility test was passed, the inactivated bacterial culture was centrifuged at 5,000 rpm for 15 min and pellet was washed twice with sterile normal saline. The bacterial concentration was adjusted to 10^9 cfu/ml using Macfarland standard and tween 80 was added at 10% to the volume of culture. Mineral oil adjuvant was prepared by thorough mixing of mineral oil, span 80 at 10% to the volume of mineral oil and it was autoclaved. The bacterial suspension was gradually added to the mineral oil adjuvant over a period of 5 min. High-speed mixing was done for 15 min to prepare the adjuvant. Inactivated oil adjuvant vaccine was prepared by mixing mineral oil, surfactants and bacterial culture in the ratio of 6:1:3. For safety checking, prepared autogenous vaccine was inoculated in mice (0.2ml s/c) and streaked on MacConkey agar plate for sterility checking.

RESULTS AND DISCUSSION

Klebsiellosis is rarely reported in rabbits, although Boucher and Nouaille (1996) have described outbreaks in France among rabbits ageing from 14–28 days of age, and Coletti *et al*. (2001) reported outbreak in rabbits in Italy. In the present study, an outbreak was reported in recently weaned animals and 20% mortality was observed in the rabbit farm. Dead and ailing rabbits and recovered rabbits are shown in Figs 3 and 4.

Necropsy revealed accumulation of mucus in the trachea, mild pneumonic changes in the lung and no observable changes in other organs. Coletti *et al*. (2001) reported mucous catarrhal enteritis and petechial hemorrhages in the cecal wall, pale liver and slightly enlarged spleen in *Klebsiella pneumoniae* infection of weaned rabbits. But, in the present outbreak, a predominant pneumonic and septicemic signs were observed with mild enteritis. The enteric forms caused in rabbits have been documented as frequent and economically important in France, Spain and Italy (Babini and Livermore 2000). Boucher and Nouaille (1996) reported *Klebsiella pneumoniae* disease in rabbit intensive farms, in which this bacterium is frequently isolated from the gastrointestinal tract of suckling rabbits, between the second and fourth weeks of age, showing a case history of diarrhoea.

In the present case, *Klebsiella pneumoniae* was isolated from heart blood swab and tracheal swab in pure culture

Fig. 1. Results of biochemical test in API 20E strips

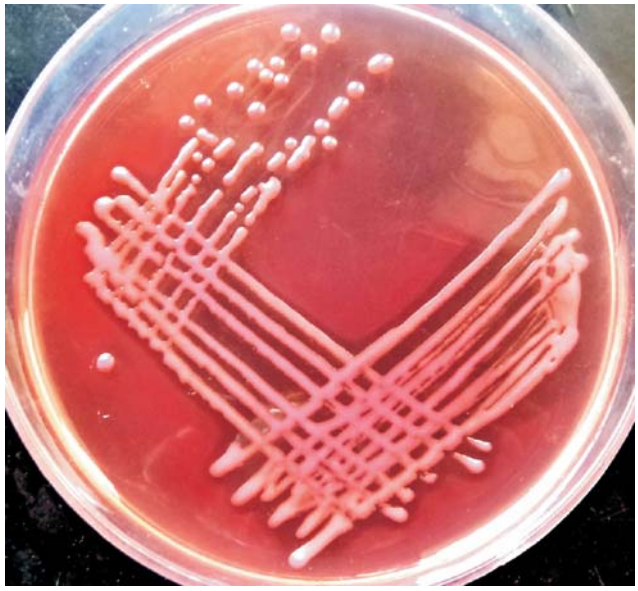

Fig. 2. Cultural charactertics of *Klebsiella pneumoniae* on MacConkey agar plate.

Table 1. Cultural, morphological and biochemical characteristics of isolated *Klebsiella pneumoniae*

Test performed	Results	Inference
Colonial morphology	Pink colonies	Mucoid lactose fermenting colonies seen
Gram stain	Gram negative	Rod to coccobacillus shaped
Negative stain	Capsule present	Capsule was observed around cell wall
Catalase production	Positive	Active bubbling observed
Oxidase production	Negative	No color change observed
H_2S production	Negative	Orangish yellow colour observed
Motility	Negative	No motility
Nitrate reduction	Positive	Red colour observed
ONPG	Positive	Yellow colour observed
Citrate utilization	Positive	Blue colour observed
Lysine utilization	Positive	Purple colour observed
Urease	Negative	Orangish yellow colour observed
Ornithine utilization	Negative	Yellow colour observed
Voges Proskauer's	Positive	Pinkish red colour observed
Methyl red	Positive	Red colour observed
Indole	Negative	No colour observed
Malonate utilization	Positive	Blue colour observed
Phenylalanine deamination	Negative	No colour observed
Esculin hydrolysis	Positive	Black colour observed
Sugar fermentation (arabinose, xylose, adonitol, rhamnose, cellobiose, melibiose, saccharose, raffinose, trehalose, glucose and lactose)	Positive	Yellow colour observed

Fig. 3. Dead and ailing rabbits

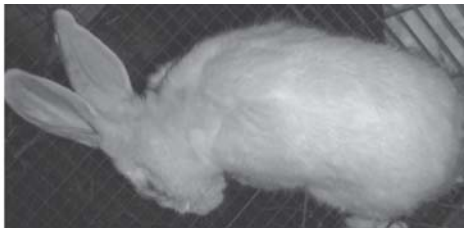

Fig. 4. Recovered rabbits.

and it was confirmed by cultural, staining and biochemical characters (Quinn *et al.* 1994). Cultural, morphological and biochemical characteristics of isolated *Klebsiella pneumoniae* are shown in Table 1. The results of biochemical test in API 20E strips are shown in Fig.1 and cultural charactertics of *Klebsiella pneumoniae* on MacConkey agar plate is shown in Fig. 2.

The antibiotic sensitivity test of isolates showed resistant to ciprofloxacin, gentamicin, oxytetracycin, streptomycin, amoxycillin, amikacin and cotrimoxazole and intermediate to enrofloxacin. The previous evidences state that *Klebsiella* are often resistant to multidrug which also implicated plasmids as the primary source of the resistance genes (Hudson *et al*. 2014). *Klebsiella* with the ability to produce extended-spectrum beta-lactamases (ESBL) are resistant to many classes of antibiotics. The most frequent resistances include resistance to aminoglycosides, fluroquinolones, tetracyclines, chloramphenicol and trimethoprim/ sulfamethoxazole (Nathisuwan *et al*. 2001).

In this case, inactivated oil adjuvant vaccine was prepared against isolated multidrug resistant *Klebsiella pneumoniae*. Oil adjuvant vaccines enhance the immune response to most antigens and prolong the duration of immunity. Mineral oil emulsions are widely used to produce a prolonged slow release of antigen from aqueous phase. They are used with antigen to potentiate the antibody response, also, to prolong their action (Cox and Coulter 1997, Aucoutuies *et al*. 2001) and also adjuvant can reduce the quantity of antigen needed to generate a protective immune response and enable the vaccine to be cheaper, they can prolong the immune response of the vaccinated animals and birds (Aucoutuies *et al.* 2001).

Vaccination is still considered as one of the major tools for controlling the disease. Hence, to control outbreak caused by multidrug resistant *Klebsiella pneumoniae* infection in rabbits autogenous vaccine was prepared and it was given to the remaining rabbits in the farm at the dose of 0.5ml s/c. The mortality and infection was reduced gradually and outbreak was completely controlled after administration of vaccine and produced noticeable protection against disease. This report is believed to be the first case report of multidrug resistant *Klebsiella pneumoniae* outbreak in rabbits in India and its control by autogenous inactivated vaccine.

ACKNOWLEDGEMENT

The authors are thankful to the Dean, Veterinary College and Research Institute, Namakkal for facilities provided for this study.

REFERENCES

Aucoutuies J, Dupuis L and Ganne V. 2001. Adjuvants designed for veterinary and human vaccines. *Vaccine* **19**: 2666–72.

Babini G S and Livermore D M. 2000. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997–1998. *Journal of Antimicrobial Chemotherapy* **45**: 183–89.

Boucher S and Nouaille L. 1996. Manuel pratique des maladies des lapins. *France Agricole éd., Paris*, 256.

Clinical and Laboratory Standards Institute. 2008. "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals," approved standard- Third edition CLSI document M31–A3, Wayne.

Coletti M, Passamonti F, Del Rossi E, Franciosini M P and Setta B. 2001. *Klebsiella pneumoniae* infection in Italian rabbits. *Veterinary Record* **149**: 20.

Cox J C and Coulter A R. 1997. Adjuvants. A. Classification and review of their modes of action. *Vaccine* **15** (3): 248–56.

Diane E Roberts, Heidi M McClain, Dennis S Hansen, Pamela Currin and Elizabeth W Howerth. 2000. An outbreak of *Klebsiella pneumoniae* infection in dogs with severe enteritis and septicemia. *Journal of Veterinary Diagnostic Investigation* **12**: 168–73.

Hudson, Corey, Bent, Zachary, Meagher, Robert, Williams and Kelly. 2014. "Resistance Determinants and Mobile Genetic Elements of an NDM-1-Encoding *Klebsiella pneumoniae* Strain". *PLoS One*. doi:10.1371/journal.pone.0099209.

Nathisuwan S, Burgess D S and Lewis J S. 2001. Extended-spectrum â-lactamases: Epidemiology, detection, and treatment. *Pharmacotherapy* **21** (8): 920–28.

Quinn P J, Carter M E, Markey B K and Carter G R. 1994. *Veterinary Clinical Microbiology.* Wolfe Publication, London, U.K. p217–19.