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Isolation, characterization and antibiotic sensitivity pattern of bacteria associated with respiratory tract diseases of pigs

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Respiratory disease has consistently been reported as one of the most important causes of both morbidity and mortality in pigs. Bacterial lung infections are a major cause of economic losses in the pig industry; they are responsible for approximately 50% of the antibiotics used in pigs and therefore, present an increasing concern to consumer protection agency (Altrock 1998). Many pathogens can act as opportunistic agents on an already compromised immune system, or can themselves become exacerbated by infection with other opportunistic pathogens. This can result in a more complicated etiology, which may be associated with higher mortality. Little is known about the aetiology of respiratory diseases of pigs in India and hence the present study was undertaken to isolate, characterize and determine the antibiogram of bacteria associated with respiratory tract diseases of pigs.

Animal and sample collection: Pigs (25) of the Centre that showed the clinical signs (such as coughing, difficulty in breathing, nasal discharge and dyspnoea) of respiratory tract disease and died of respiratory tract problems (5) were used for the study. All affected pigs were within 7–11 months of age. The study was carried out during the period of June 2009 to May 2010. The nasal samples were collected by inserting sterile cotton-tipped applicator sticks into the nasal passageways after proper cleaning and disinfection of external nares. Using sterile scissors and tissue forceps, pieces of lung from the dead animals were collected separately into sterile screw-capped universal bottles and transported in a cool box for further processing.

Isolation and identification of bacteria: To isolate the bacteria from nasal swabs and lung tissues, general and selective agars such as blood agar base, nutrient agar and Mac Conkey agar were used. The plates were incubated aerobically at 37°C for 24–48 h (Carter 1984). After taking note of cultural characteristics, positive cultures were subjected to Gram's staining. Mixed colonies and gram-negative bacteria were sub-cultured on both blood and McConkey agars and incubated aerobically for further 24 h. Pure cultures of single colony type, from both blood and McConkey agars were transferred onto nutrient agar-slants for a series of biochemical tests including catalase, oxidase and fermentative/oxidative tests for final identification following standard procedures (Carter 1984). *Streptococcus suis* was identified as per Tarradas *et al.* (1994).

Antibiotic sensitivity test: In-vitro antibiogram of isolated bacteria were tested by the disc diffusion technique according to Bauer *et al.* (1966) using 14 different antimicrobial discs. The antimicrobial discs used were amikacin, ceftriaxone, cephalexin, chloramphenicol, chlortetracycline, ciprofloxacin, doxycillin, enrofloxacin, gentamicin, kanamycin, penicillin G, streptomycin, sulphadiazine and tetracycline.

The clinical signs shown by the pigs affected with respiratory diseases were coughing, sneezing, abdominal type of breathing and dyspnoea. Bleeding from nose was also observed in 3 cases. The present finding is almost similar with the findings of Choi *et al*. (2003) who also observed similar clinical signs in pigs affected by respiratory diseases. The bacteria isolated from the lung tissues and nasal swabs of pigs affected with respiratory diseases were *Pasteurella multocida* (8), *Bacillus subtilis* (2), *Streptococcus suis* (5), *Staphylococcus aureus* (4), *Klebsiella pneumoniae* (3) and combined infection of *Klebsiella pneumoniae* and *Micrococcus* spp. (3). This study has shown that a variety of bacterial agents is responsible for respiratory problems in pigs. Several workers (Ravishankar *et al*. 2005, Dutta *et al.* 2007) reported isolation of similar bacteria from pneumonic lungs of pigs. The invariable isolation of these organisms from the pneumonic lungs of pigs might indicate their role in different respiratory syndromes.

All *Pasteurella multocida* (*P. multocida*) isolates in the present study showed positive reaction to nitrate reduction test, indole test, oxidase, catalase, glucose, mannitol and sucrose fermentation tests except for 2 isolates which showed

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Table 1. Antimicrobial sensitivity pattern of bacteria isolated from pigs with respiratory diseases

Antimicrobials	*P. multocida*	*S. aureus*	*S. suis*	*Bacillus subtilis*	*K. pneumoniae*	*Micrococcus* spp.
Amikacin	7 (87.50)	4 (100.00)	5 (100.00)	2 (100.00)	6 (100.00)	3 (100.00)
Ceftriaxone	6 (75.00)	1 (25.00)	0 (0.00)	2 (100.00)	0 (0.00)	0 (0.00)
Cephalexin	0 (0.00)	1 (25.00)	0 (0.00)	2 (100.00)	0 (0.00)	0 (0.00)
Chloramphenicol	8 (100.00)	4 (100.00)	5 (100.00)	2 (100.00)	6 (100.00)	0 (0.00)
Chlortetracycline	8 (100.00)	1 (25.00)	4 (80.00)	2 (100.00)	6 (100.00)	3 (100.00)
Ciprofloxacin	8 (100.00)	2 (50.00)	4 (80.00)	2 (100.00)	6 (100.00)	0 (0.00)
Doxycillin	8 (100.00)	2 (50.00)	3 (60.00)	1 (50.00)	2 (33.33)	0 (0.00)
Enrofloxacin	8 (100.00)	3 (75.00)	5 (100.00)	2 (100.00)	6 (100.00)	3 (100.00)
Gentamicin	8 (100.00)	4 (100.00)	5 (100.00)	2 (100.00)	6 (100.00)	3 (100.00)
Kanamycin	3 (37.50)	3 (75.00)	3 (60.00)	2 (100.00)	0 (0.00)	0 (0.00)
Penicillin G	0 (0.00)	0 (0.00)	5 (100.00)	0(0.00)	0 (0.00)	0 (0.00)
Streptomycin	3 (37.50)	1 (25.00)	3 (60.00)	2 (100.00)	1 (16.66)	0 (0.00)
Sulphadiazine	0 (0.00)	0 (0.00)	0 (0.00)	0(0.00)	6 (100.00)	0 (0.00)
Tetracycline	5 (62.50)	0(0.00)	0 (0.00)	0(0.00)	1 (16.66)	0 (0.00)

Figures in the parenthesis indicate % susceptibility.

negative reaction to nitrate reduction test. It was also observed that all isolates were negative to lactose fermentation and citrate utilization test. All the isolates of *Bacillus subtilis* (*B. subtilis*) were positive for oxidase production, citrate utilization, nitrate reduction and sucrose fermentation test. All the isolates of *S. suis* showed positive reaction to trehalose and hydrolysis of esculin and negative reaction to growth in 6.5% NaCl and Voges Proskauer (VP) test. Similar biochemical properties of *S. suis* have also been reported by Tarradas *et al*. (1994). All the isolates of *Staphylococcus aureus* were found positive for oxidase, catalase and coagulase production and could ferment glucose, mannitol, lactose, sorbitol and sucrose. The study also revealed that all the isolates of *Klebsiella pneumoniae* were positive for catalase, VP, glucose, sucrose, citrate utilization and nitrate reduction tests whereas they were negative for methyl red, indole and H_2S production. Two isolates of *K. pneumoniae* were found to be negative for urease test whereas rest 4 isolates were found positive for the same.

The antibiotic sensitivity pattern of bacteria isolated from pigs with respiratory diseases is given in Table 1. All the isolates of *P. multocida* were found sensitive to chloramphenicol, chlortetracycline, ciprofloxacin, doxycillin, enrofloxacin and gentamicin whereas isolates were found to be resistant to cephalexin, penicillin G and sulphadiazine. Similar finding was also reported by Dev Sharma *et al*. (2004). Hormansdorfer and Bauer (1998) reported that over 90% of porcine *P. multocida* were found sensitive to enrofloxacin and chloram-phenicol. The present study corroborates with the findings of Riesen and Perreten (2009) who also observed higher sensitivity of *S. aureus* isolates from pigs to antimicrobials such as chloramphenicol and gentamicin and lowest sensitivity to antimicrobials such as penicillin and tetracycline. As in the present study, Vela *et al.* (2005) recorded higher sensitivity of *S. suis* isolates to enrofloxacin, gentamicin and penicillin and resistance to terracycline and sulphadiazine. It was also observed that all the isolates of *B. subtilis* were sensitive to majority of the antimicrobials tested in the study which also corroborated with the findings of Coonrod *et al.* (1971). From the study it was also observed that both *K. pneumoniae* and *Micrococcus* species were highly sensitive to amikacin, chlortetracycline, enrofloxacin and gentamicin.

SUMMARY

The study was conducted to isolate the aerobic bacterial pathogens associated with respiratory diseases of pigs. The bacteria isolated from the pigs affected with respiratory diseases were *Pasteurella multocida*, *Bacillus subtilis*, *Streptococcus suis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Micrococcus* spp. All the isolates of *P. multocida* were found sensitive to chloramphenicol, chlortetracycline, ciprofloxacin, doxycillin, enrofloxacin and gentamicin whereas isolates were resistant to cephalexin, penicillin G and sulphadiazine. The isolates of *Staphylococcus aureus* showed highest sensitivity to amikacin, chloramphenicol and gentamicin and lowest sensitivity to penicillin, sulphadizine and tetracycline. Isolates of *Streptococcus suis* showed higher sensitivities to amikacin, enrofloxacin, gentamicin and penicillin and resistance to ceftriaxone, cephalexin, terracycline and sulphadizine. All the isolates of *Bacillus subtilis* were sensitive to majority of the antimicrobials tested in the study and *Micrococcus* species were highly sensitive to amikacin, chlortetracycline, enrofloxacin and gentamicin

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