

Isolation, characterization, antibiogram and pathology of *Pasteurella multocida* isolated from pigs

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Abstract

Aim: Isolation, characterization and antibiogram of *Pasteurella multocida* from diseased pigs of district Durg of Chhattisgarh, and to study pathological changes caused by swine pasteurellosis.

Materials and Methods: An outbreak of swine pasteurellosis was suspected in pigs of Ruwabandha (Bhilai), Anjora, Somni, Tedesara, Tirgajhola villages of Durg district in Chhattisgarh, India during August and September of 2011. Nasal Swabs and blood samples from ailing pigs and heart blood and impression smears from morbid pigs were processed for detection and isolation of *P. multocida* by bacteriological methods. Detailed necropsy was conducted and gross and histopathological lesions were recorded. The test Isolates were subjected to antimicrobial sensitivity profile by disc-diffusion method.

Results: The blood smears from heart blood and tissue impression smears revealed teaming of bipolar organisms indicating the presence of *Pasteurella spp.* The isolates obtained were subjected to Gram's staining for checking the purity and bipolar morphology and characterized biochemically. Gross lesions included severe acute pneumonia and haemorrhages in lungs, petechial haemorrhages on serous membranes and other visceral organs. On histopathological examination, lungs showed typical fibrinous bronchopneumonia, multifocal suppuration. All the isolates of *P. multocida* were 100% sensitive to Amoxicillin, Gentamicin, Enrofloxacin and showed 100% resistance to Ceftizoxim and Cloxacillin.

Conclusion: Gross and microscopic lesions in dead animals are of great diagnostic value and are of characteristic of *P. multocida* infection. Cultural, morphological and biochemical characters are useful to rule out the causative agent as *P. multocida*. Antibiotic sensitivity pattern of the isolates should routinely be carried out for knowing the antibiotic resistance trends in an endemic area.

Keywords: Chhattisgarh, *Pasteurella multocida*, pathology, pneumonic pasteurellosis, swine pasteurellosis.

Introduction

Pasteurella multocida is a commensal and pathogen of respiratory tract of animals. Haemorrhagic Septicaemia is an acute infection of cattle, buffaloes, sheep and goats, caused by *P. multocida* capsular type B and occasionally by D and E. In swine, capsular types A and D are most often associated with atrophic rhinitis, while type A are associated with pneumonia, pleuritis and abscessation [1]. The disease occurs mainly in South East Asian countries and also in Africa, where it causes very heavy death losses. Haemorrhagic septicaemia is one of the most important bacterial diseases of cattle, buffalo and pigs in India. The disease occurs mainly during the rainy season. It spreads rapidly among groups of animals, causing morbidity and mortality between 50 to 100 percent. Pneumonic pasteurellosis is characterized by chronic pneumonia, purulent bronchopneumonia and pleurisy [2]. Affected pigs have fever of up to 106°F, are anorectic and disinclined to move. They show significant respiratory

distress, often breathing through the mouth. Death is a common phenomenon after a clinical course of 4-7 days. There is a marked tendency for the disease to become chronic, resulting in reduced weight gains and frequent relapses. On post mortem examination there is a chronic bronchopneumonia with abscessation. Pleuritis is common and there may also be pericarditis. Peracute cases show an acute necrotizing fibrinous bronchopneumonia. Septicaemic disease with death occurring within 12 hours and without signs of pneumonia is sometimes observed in baby pigs. The disease occurs in all ages of pigs including adults and is manifested by fever, dyspnoea and congestion on serosal surfaces [3].

Chhattisgarh state of India is very rich in its live-stock wealth with twelve million seven hundred thousand animals against twenty-five million five hundred thousand human population. Cattle population is the highest with 64%, followed by goats (16%), buffaloes (14%) and sheep and pigs being the lowest (6%). Livestock in Chhattisgarh are smaller in size with poor production potentialities, due to poor genetic potential coupled with inadequate availability of feed and fodder [4]. Pig production in Chhattisgarh is invariably a small-scale, backyard, marketed-oriented enterprise. It

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Figure-1: The photograph showing a case of oedematous swelling in the pharyngeal and ventral cervical region and brisket of adult Hampshire crossbred pig and subsequent scratch wound.

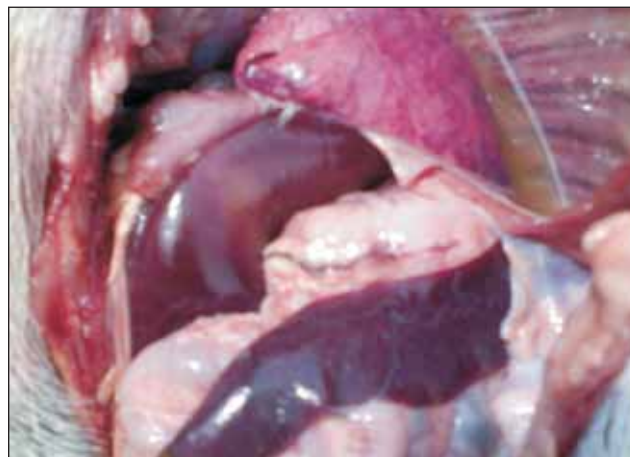


Figure-2: The photograph showing consolidation of lungs, hydrothorax, congested liver and splenomegaly of pig died of swine pasteurellosis

is practised mainly by Scheduled Tribes (ST) and some Other Backward Classes (OBC) to generate income, accumulate capital and fulfil socio-cultural obligations. Pigs are still considered scavenging animals and that the underprivileged are involved in pig production [5].

Swine Pasteurellosis and haemorrhagic septicaemia are controlled by vaccination of animals with H.S. vaccine. The vaccines preparation used for cattle buffaloes and pigs are same. The vaccinated and non-vaccinated animals were affected during each outbreak of swine pasteurellosis in Chhattisgarh with variable mortality rates and different disease manifestations. It indicates that there is possibility of variation in antigenicity between vaccine strain and field isolate of *Pasteurella*. Therefore, there is urgent need of bacteriological and pathological characterization the potential pathogen *P. multocida* involved in recurrent outbreaks.

Materials and Methods

Case description: An outbreak of swine pasteurellosis was suspected in pigs of Ruwabandha (Bhilai), Anjora, Somni, Tedesara, Tirgajhola villages of Durg district in Chhattisgarh state of India during August to September of 2011. The villages are located within 30 kilometres of radius. Altogether, there were around 50 pig rearing families having 5-30 pigs per family. A total number of 377 pigs were under risk of the disease, out of which 249 animals were died. A total number of 126 animals were attended for necropsy and the representative tissue samples were collected for histopathological and bacteriological examination. The overall mortality rate was 66%. The affected animals were off fed and had high fever (41-42°C). The atmospheric temperature and humidity were recorded between 24.5°C - 29.5°C and 76% - 93% respectively. Socio-economically poor families used to rear pigs under backyard system and housed in poorly managed muddy houses. These animals were fed with agricultural and kitchen waste and were allowed to graze in fields. Pig farmers had little knowledge of pig diseases and health care management. Outbreak of disease caused high mortality within all age group of

animals. Clinical signs exhibited by ailing adult pigs were pyrexia (41-42°C), staggering gait, dullness, serous nasal discharge and dyspnoea leading to death after a clinical course of 4-6 days. Case fatality rate was 95% in adult animals and 100% in piglets. Infected piglets showed high fever and serous nasal discharge and died within 24 hours of onset of fever. A very few pigs were treated with Enrofloxacin. Atypical cases of oedematous swellings noted in the pharyngeal region, these swellings spread to the ventral cervical region and brisket of adult Hampshire crossbred pigs. Later, the edematous part was scratched by affected pigs and ulcerated wound followed (Figure-1).

Processing of samples

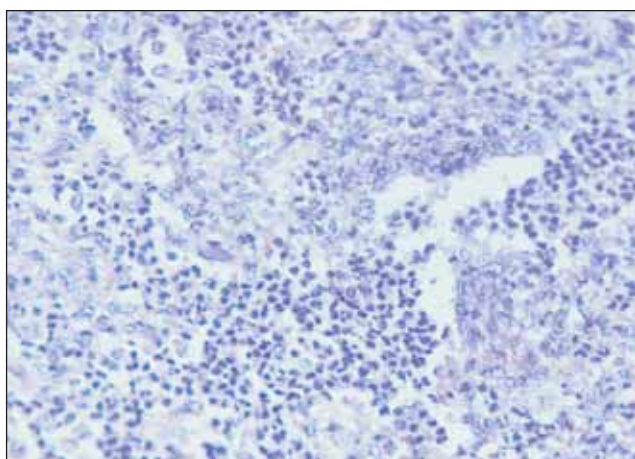
Pathological Studies: The infected pigs were examined clinically. Detailed necropsy was conducted and gross lesions were recorded. Tissue samples from heart, lungs, liver, spleen, kidneys and Lymph nodes were collected in 10% Formal saline solution for histopathological studies. Morbid tissue samples then processed by routine histopathological techniques and stained with haematoxylin and eosin stains [6].

Bacteriological Isolation: The blood smears were prepared from heart blood and the impression smears were prepared from Lungs, spleen and stained with Methylene Blue and Leishman's stain to demonstrate the causative organisms. Heart blood, nasal swabs and tissue samples from lungs and spleen were collected and processed for bacteriological isolation. The clinical samples for bacteriological examination were collected by using sterile HiMedia Transport swabs (HiMedia Laboratories Pvt. Ltd., Mumbai, India).

For primary isolation of the organism the samples were inoculated on Sheep Blood agar (SBA) and Mac Conkey agar (MLA) according to methods described by Cruickshank *et al.* [7] and Quinn *et al.* [2] The pure cultures were transferred to Brain Heart Infusion (BHI) Agar slants for further identification. *P. multocida* isolates were identified by biochemical tests *viz.*, Oxidase, Catalase, Indole, Citrate utilization, Nitrate reduction

Table-1: Showing the antibiotic sensitivity pattern of *P. multocida*

Antibiotic	% of sensitive isolates	% of resistant isolates	% of intermediate isolates
Ceftizoxim(Ck)	0	100	0
Amoxicillin (Am)	100	0	0
Cloxacillin (Cx)	0	100	0
Roxithromycin (Ro)	4.3	95.6	0
Oxacillin (Ox)	0	100	0
Amikacin (Ak)	91.3	0	8
Gentamicin (G)	100	0	0
Cephalexin (Cp)	0	100	0
Trimethoprim (Tr)	100	0	0
Cotrimoxazole (CoT)	78.2	0	21.7
Oxytetracycline (O)	100	0	0
Enrofloxacin (Ex)	100	0	0
Chloramphenicol (C)	100	0	0
Sulphadiazine (Sz)	100	0	0
Ofloxacin (Of)	100	0	0

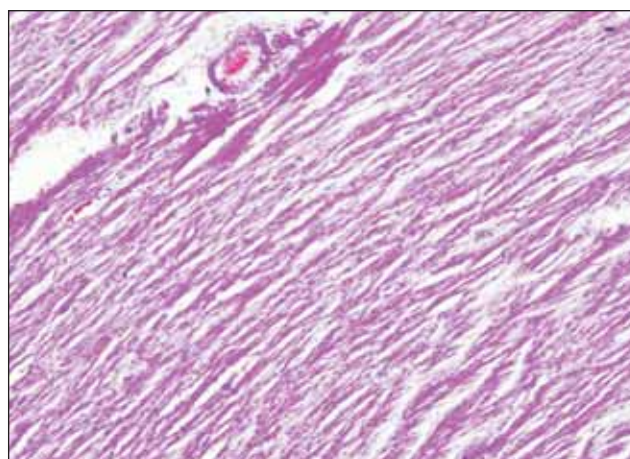
**Figure-3:** Microphotograph showing fibrinous exudate and flooding of polymorphonuclear cells in alveoli.

and fermentation of sugars *viz.*, glucose, mannitol, sucrose, mannose, maltose, arabinose, lactose, dulcitol, salicin, inositol and trehalose as per methods described by Cowan and Steel [8].

Antimicrobial drugs sensitivity pattern of *P. multocida*: All the isolates were tested for sensitivity pattern against 15 different antimicrobial agents (Table-1) commonly used in field practices. Antibiotic discs from HiMedia Laboratories Pvt Limited, Mumbai (India) were used for in-vitro antibiotic sensitivity test as per the method described by Bauer et al. [9]. The diameters of inhibition zones surrounding the antibiotic discs were measured and subsequently matched with the standard inhibition zone diameters of respective antibiotic discs. On the basis of size of inhibition zones of various antibiotics, the isolates were classified as sensitive, intermediately sensitive or resistant.

Results

Gross and histopathology: On post-mortem examination, Petechiae and frothy exudate were present in trachea and bronchi. Petechiae were seen all over the serous membranes and peritoneum. There was hydrothorax with accumulation of straw coloured fluid. Endocarditis was observed in the heart. Lungs showed severe consolidation along with the presence of multiple focal haemorrhages. Spleens from most of the dead pigs were very much enlarged (Figure-2).

**Figure-4:** Microphotograph showing presence of thrombi in blood vessel of heart

Liver and kidneys were swollen and contained petechial haemorrhages. Lymph nodes were enlarged, oedematous and haemorrhagic.

Microscopically, lungs showed typical fibrinous bronchopneumonia, multifocal suppuration, septae were thickened with fibrin, combined with cellular infiltration and edema. Alveoli were filled with fibrinous exudate, erythrocytes and polymorphonuclear cells (Figure-3). Pleura were severely thickened. There were sub-pleural haemorrhages. Heart of some pigs showed presence of thrombi, haemorrhages and necrosis of myocardium (Figure-4). Glomeruli and kidney tubules showed haemorrhages and necrotic changes. Severe congestion and haemorrhages were observed both in cortex and medulla of lymphnodes. There were haemorrhages and necrosis in Liver.

Isolation and characterization of *P. multocida*: The blood smears from heart blood and tissue impressions revealed teeming number of bipolar organisms indicating the presence of *Pasteurella* spp. The organisms were isolated from morbid tissue samples *viz.* lungs, heart blood and spleen as well as clinical blood/nasal samples of the pigs. Clinical samples, on sheep blood agar plates yielded tiny transparent, non-hemolytic colonies after an incubation of 24 hours at 37°C (Figure-5a). Subsequently, the non-haemolytic single colony from SBA was transferred to MLA plate and incubated at 37°C for 24 hours. The isolates which



Figure-5a: Photograph showing non-hemolytic smooth colonies of *P. multocida* on SBA plate.



Figure-5b: Microphotograph showing *P. multocida* as coccobacillary bipolar rods.

failed to grow on MLA were preliminary presumed to be *P. multocida*. Single non-haemolytic colony of these isolates was picked up from primary culture and re-streaked on fresh SBA plate and incubated at 37°C for 24 hours to obtain single colony of pure culture of the isolates. On the basis of Gram staining, the isolates were Gram's negative, cocco-bacillary rods (Figure-5b). The isolates so obtained were confirmed by biochemical characterization. In the present study, the test isolates were found to be positive for oxidase, catalase, indole production and reduction of nitrate. The test isolates fermented dextrose, trehalose, xylose and mannitol. The isolates failed to ferment lactose, arabinose and dulcitol.

Antimicrobial sensitivity test: The test isolates were found 100% sensitive to Amoxycillin, Gentamicin, Enrofloxacin, Sulphadiazine, Ofloxacin, Trimethoprim and Amikacin (91.3%), Cotrimoxazole (78.2%). Whereas, the isolates showed 100% resistance against Ceftizoxim, Cephalexin, Cloxacillin and 95.6% resistance was shown against Roxithromycin. The results of *in vitro* antibiotic sensitivity were interpreted and are depicted in Table-1.

Discussions

P. multocida is considered a commensal organism in the upper respiratory tract and tonsils and causes disease outbreaks in swine, cattle, buffalo, sheep, and goats under extreme environmental conditions. This organism is the most common pathogen isolated from pigs housed under poor husbandry conditions, eg, overcrowding and poor ventilation. Source of infection in recent outbreak was introduction of a sick pig from slaughter market of Bhilai. In the present study, case fatality rate was 95% in adult pigs and 100% in piglets. The high mortalities observed in many outbreaks of pasteurellosis in Chhattisgarh and other parts of India [2, 10-13]. Similar outbreak of swine pasteurellosis in pig herd has been reported earlier with high mortality and variable degree of ulcerative skin lesions [11,14]. The ulcerative skin lesions were absent in pigs from recent outbreak but oedematous swellings were noted

in the pharyngeal region of some of the infected pigs which is the predominant manifestation in cattle and buffaloes. The oedematous swellings in the pharyngeal region of diseased pigs were unique finding during this study. *P. multocida* type B:2, is responsible for haemorrhagic septicemia in dairy cattle and buffalo and many outbreaks are reported every year from all over India, suggesting that this serotype may have transmitted between bovine species and swine during recent outbreak [11,12,15]. The outbreak of pasteurellosis is attributed to impairment of host defences mechanism, strain and virulence of causative organism and various other physiological and environmental stress factors [8]. Thus, due to highly contagious nature of the disease and high mortality rate, the disease has played a major role in huge economic loss in rural population of Chhattisgarh.

Post-mortem findings, pathological changes and demonstration of bipolar organisms in impression smears suggestive of swine pasteurellosis have also been reported by other workers [11,13,16]. Broncho-pneumonia with abscessation and pleuritis have been found to be common lesions of pneumonic pasteurellosis in pigs [2,11,14]. Fibrinous and suppurative broncho-pneumonia with focal areas of necrosis typical of pneumonic pasteurellosis was found. Similar lesion were recorded by Parveena *et al.* [16].

On blood agar plates, the colonies of *P. multocida* isolate were non-hemolytic, round, smooth or mucoid. The isolate failed to grow on MacConkey's agar. On the basis of Gram's staining, the isolates were found to be Gram negative and cocco-bacillary rods in morphology. The test isolates of *P. multocida* organisms were confirmed by biochemical characters. These findings are similar to the findings of Sharma *et al.* [17], Kalorey *et al.* [12], Quinn *et al.* [2], and Jogi [14]. In the present study, the isolates were found to be positive for oxidase, catalase, indole production and reduction of nitrate. The test isolates fermented glucose and sorbitol but failed to ferment lactose, arabinose and adonitol. This is in accordance with the finding of Verma [18] and Jogi [13]. Rajni *et al.* [19] recorded that most of the

avian isolates ferment arabinose while mammalian isolates failed to ferment arabinose. The variability observed in fermentation reactions of carbohydrates might be due to geographical variation of isolates and use of chemotherapeutic agents, as these factors influence the enzyme profiles of microbes. [13, 19].

The majority of test isolates were found to be 100% sensitive to Amoxicillin, Gentamicin, Enrofloxacin, Sulphadiazine, Ofloxacin, Trimethoprim, Chloramphenicol and Oxytetracycline. Higher efficacy of enrofloxacin and ofloxacin along with chloramphenicol has also been reported by Sharma *et al.* [15] and Varte *et al.* [14]. Enrofloxacin and chloramphenicol were found to be quite effective against pasteurellosis by several research groups [14, 20]. The isolates in this study were 100% resistant to Cephalexin. Balakrishnan *et al.* [21] recorded that *P. multocida* were resistant to cephalexin. Varte *et al.* [15] and Jogi [14] recorded 100% sensitivity for Cephalexin. Rajkhowa *et al.* [22] recorded the *P. multocida* isolates from pigs were resistant to Cephalexin and Sulphadiazine. The resistance for Cephalexin in the present study could be due to development of resistance towards Cephalexin. Thus, it shows the necessity of *in vitro* antibiotic sensitivity prior to treatment.

Conclusion

There was an outbreak of swine pasteurellosis in pigs of different villages in Chhattisgarh with a case fatality rate of 90% in adult pigs and 100% in piglets. Although there are recurrent outbreaks of swine pasteurellosis in Chhattisgarh state but the clinical manifestations varies in each outbreak. It is observed in last one decade that there is a shift in antibiotic sensitivity pattern of *P. multocida*. The isolates observed resistance against conventional antibiotics particularly to Cephalexin, The same drug was found to be 100% sensitive during previous outbreaks which are leading to problems in treating the affected animals. This warrants the need for the thorough clinical and microbiological investigation of *P. multocida* in each outbreak. Furthermore, the results of this study emphasize the importance of *in vitro* antibiotic sensitivity test prior to initiate treatment.

Authors' contributions

MT: Collection of Samples and processing for isolation, characterization and antibiotic sensitivity test of *P. multocida*. Histopathological examination and manuscript writing. RCG: Planning of research methodology, technical support, histopathological examination and editing of manuscript. PM: Planning of research methodology, technical support and editing of the manuscript. All authors read and approved the final manuscript. BKC and PT: Collection and processing of samples for bacteriology and histopathology. DKN: Processing of samples for biochemical and molecular characterization. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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