# CHARACTERISTICS OF *PASTEURELLA MULTOCIDA* ISOLATED FROM HUMANS, SWINE AND POULTRY IN THAILAND

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**Abstract.** *Pasteurella multocida* is a pathogen of animals and humans. Most of the patients have been associated with animals but many cases had not contacted them. The failure to diagnose *P. multocida* infections is mostly due to misidentification on gram stained smears and inadequate laboratory identification techniques. In order to compile detailed characteristics of the organism we studied the physical and biochemical properties of 70 isolates of *P. multocida* - 17 human, 23 swine and 30 poultry. All isolates produced catalase, oxydase, indol, nitrate reduction and ornithine decarboxylase. They failed to produce urease, gelatinase, methyl red, acetoin and could not grow on MacConkey agar, SS-agar, in nutrient broth with 0% or 6% NaCl. With respect to fermentable sugars, all isolates consistantly produced acid from glucose, mannitol and mannose. None of the cultures fermented lactose, maltose and dulcitol. Marked variations in the patterns of fermentation of arabinose and xylose were found. The characteristics tested are important to facilitate identification of *P. multocida* but could not be used to differentiate the host of the bacterium.

## INTRODUCTION

*Pasteurella multocida* is a pathogenic bacterium which causes severe diseases in mammals and avians (Blood *et al,* 1983 ; Rhoades and Rimler, 1988). There have been several reports of human infections with *P. multocida.* This organism is a common cause of infection following animal bites or scratches which are mostly caused by cats or dogs (August, 1990; Norman *et al,* 1971; Vakil *et al,* 1985). It causes the typical clinical manifestations of a developing cellulitis at the site of injury. The infections were potentially dangerous and can cause a chronic local infection of deep tissues and septicemia (Francis *et al,* 1975; Hubbert and Rosen, 1970a). *P. multocida* can be isolated as a commensal organism from the respiratory tract of patients with underlying pulmonary disease, but serious respiratory tract infections including pneumonia, empyema and lung abscesses may develop (Weber *et al,* 1984). *P. multocida* often acts as an opportunistic pathogen and causes bacteremia in patients with liver dysfunction, septic arthritis in damaged joints, meningitis in the very young and elderly and pulmonary colonization or invasion in patients with underlying respiratory tract abnormalities. *P. multocida* is also a pathogen of systemic infections including meningitis, brain abscess, spontaneous bacterial peritonitis, intra-abdominal abscess (Weber *et al,* 1984), urinary tract infection (Hubbert and Rosen, 1970b) and renal abscess (Brasfield, 1978; Dixon and Keresteci, 1967). Neonatal infections have also been reported and the patients died of sepsis due to *P. multocida* within the first 72 hours of life (Bate *et al,* 1965; Strand and Helfman, 1971).

Domestic animals are a potential threat to patients with cirrhosis especially if close contact with the animal occurs (Szpak *et al,* 1980; Vakil *et al,* 1985). Isolation of identical biotypes from both

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animal and patient in the case of animal exposure suggests that the animal is the source of infection (Vakil *et al,* 1985).

Identification of the organisms is mainly based on their biochemical properties as substrates particularly fermentative reactions using various carbohydrates as assayed by conventional methods (Clark *et al,* 1984; Heddleston, 1975; Weaver *et al,* 1985). Rapid test systems, eg API 20 E, Minitek and Oxi/Ferm systems to identify *P. multocida* have been attempted (Collins *et al,* 1981), but the identification of *P. multocida* using the rapid test system was unsatisfactory. Several studies have been reported on the physical and biochemical properties of *P. multocida.* They found that different sources of the cultures showed marked variation, especially in fermentation reactions of carbohydrates (Chandrasekaran *et al,* 1981; Dorsey, 1963; Heddleston, 1976; Heddleston and Wessman, 1975). This variation may lead to misidentification.

In Thailand, Jayanertra *et al* (1978) reported a case of *P. multocida* infection in human with liver cirrhosis. They stated that misidentification of the organism was one of the important reasons which led to the failure of human pasteurellosis detection. Most medical bacteriologists are unfamiliar with this organism and therefore many identify it something else. On the contrary, animal infections such as fowl cholera in avians, and hemorrhagic septicemia in cattle have been more throughly investigated because of their economic importance.

As mentioned earlier, human infections occur in individuals closely associated with animals and the identification of human pasteurellosis is still a problem. To assist bacteriologists to recognize *P. multocida* isolated from both humans and animals, we compare the physical and biochemical properties of this bacterium from various sources.

## MATERIALS AND METHODS

### Tested cultures

Seventy isolates of *P. multocida,* 17 human, 23 swine and 30 poultry (19 duck and 11 chicken), were use in this study. The organisms were isolated from patients and infected animals in Thailand. All strains were characterized with regard to capsular and somatic serotypes (Unchitti *et al,* unpublished).

### Physical and biochemical tests

Cultures were purified and propagated on trypticase soy agar (Difco) with the addition of 5% sheep blood (BTSA). Hemolysis and colony morphology were observed on BTSA after incubated at 37°C for 24 hours. Capsular and gram stains were carried out as described by Jasmin (1945) and Cowan (1974), respectively. Growth on MacConkey agar and SS agar was determined at 37°C after 24 hours incubation. Salt tolerance was assessed by inoculation of young cultures into nutrient broth without salt and with 6% NaCl. Ability to grow at different temperatures was tested in brain heart infusion broth (BHI) incubated at 25°C, 37°C and 42°C for 24 hours. Motility was determined in semisolid agar and in OF medium (Cowan, 1974). Catalase, cytochrome oxidase, urease, nitrate reduction, production of indol, methyl red, acetoin and gelatinase, esculin hydrolysis and utilization of citrate were determined according to conventional biochemical tests (Clark *et al,* 1984; Cowan, 1974). The carboxylase test was carried out in decarboxylase medium containing 1% arginine, lysine and ornithine (Clark *et al,* 1984). The fermentative oxidative reaction was performed by stabbing 2 tubes of 1% glucose OF medium as described by Clark *et al* (1984). Fermentation reactions were determined in phenol red broth base containing 1% glucose, lactose, maltose, mannitol, mannose, sucrose, arabinose, xylose and dulcitol. Two drops of young culture in BHI were inoculated into the medium and incubate for 24 hours, 2 and 7 days at 37°C.

## RESULTS

All cultures produced glistening, smooth, entire edge, convex colonies with no hemolysis. The size of colonies varied from 0.5 to 2.5 mm. They were small gram negative coccobacilli. They produced capsules which were seen as halos around the cells by gram staining or by the Jasmin method.

Table 1 shows the physical and biochemical reactions of 70 isolates of *P. multocida.* Isolates from humans, swine, poultry (duck and chicken)

Table 1

Biochemical and physiological characteristics of 70 strains of *P. multocida* isolated from human, swine and poultry.

| Characteristics | Human (17) | Swine (23) | Poultry (30) | % of total (70) |
|---|---|---|---|---|
| Hemolysis | 0 | 0 | 0 | 0 |
| Capsule | 17* | 23 | 30 | 100 |
| Growth on : | | | | |
| MacConkey | 0 | 0 | 0 | 0 |
| SS agar | 0 | 0 | 0 | 0 |
| Motility | 0 | 0 | 0 | 0 |
| Catalase | 17 | 23 | 30 | 100 |
| Oxidase | 17 | 23 | 30 | 100 |
| Citrate (Simmon) | 0 | 0 | 0 | 0 |
| Urease | 0 | 0 | 0 | 0 |
| Nitrate reduction | 17 | 23 | 30 | 100 |
| Indol | 17 | 23 | 30 | 100 |
| Methyl red | 0 | 0 | 0 | 0 |
| Voges-Proskauer | 0 | 0 | 0 | 0 |
| Gelatinase | 0 | 0 | 0 | 0 |
| Esculin hydrolysis | 0 | 0 | 0 | 0 |
| Lysine dihydrolase | 0 | 0 | 0 | 0 |
| Arginine dihydrolase | 0 | 0 | 0 | 0 |
| Ornithine decarboxylase | 17 | 23 | 30 | 100 |
| Nutrient broth, 0% NaCl | 0 | 0 | 0 | 0 |
| Nutrient broth, 6% NaCl | 0 | 0 | 0 | 0 |
| Growth on : | | | | |
| 25°C | 17 | 23 | 30 | 100 |
| 37°C | 17 | 23 | 30 | 100 |
| 42°C | 13 | 22 | 30 | 93 |
| Fermentative or oxidative | F | F | F | F,100 |
| Gas from glucose | 0 | 0 | 0 | 0 |
| Acid from : | | | | |
| Glucose | 17 | 23 | 30 | 100 |
| Lactose | 0 | 0 | 0 | 0 |
| Maltose | 0 | 0 | 0 | 0 |
| Mannitol | 17 | 23 | 30 | 100 |
| Mannose | 17 | 23 | 30 | 100 |
| Sucrose | 17 | 20 | 30 | 96 |
| Arabinose | 5 | 1 | 24 | 43 |
| Xylose | 11 | 23 | 3 | 53 |
| Dulcitol | 0 | 0 | 0 | 0 |

Figures in parentheses indicate numbers of strains tested.
F = fermentative * Number of strains positive.

varied only in the fermentation of sucrose, arabinose and xylose, and the ability to grow at 42°C. Four strains isolated from humans and 1 from swine could not grow at 42°C, 3 swine isolates failed to ferment sucrose. Twenty-nine, 4 and 80% of human, swine and poultry isolates, respectively, produced acid from arabinose. Sixty-five, 100 and 10% of strains isolated from humans, swine, and poultry fermented xylose.

## DISCUSSION

Heddleston (1976) demonstrated variation in fermentation activity of *P. multocida* from various sources. He concluded that this property could not be used to indicate the host origin of the isolates. Strains from humans expose to different animals showed differences in fermentation of lactose, arabinose, mannitol, sorbitol, glycerol, dulcitol, trehalose and maltose (Heddleston, 1976; Holmes and Brandon, 1965; Oberhofer, 1981; Smith, 1959). In the present study, we found that the strains from avian hosts and swine were different in the fermentation of arabinose and xylose. Most avian isolates, from chickens and ducks, produced acid from arabinose (83% positive) but not from xylose (10%) while swine isolates fermented xylose (100%) but not arabinose (4%). Human strains exhibited variation in the fermentation of xylose (65%) and arabinose (29%). For purposes of comparison the data in Table 2 demonstrate similar fermentation activity with respect to arabinose and xylose of *P. multocida* strains from human and animals reported by other workers. It is worth nothing that most of our avian strains were arabinose positive and xylose negative and have been serotyped as A:1 (Unchitti *et al,* unpublished). These results support those of Heddleston (1976) who observed that serotype 1 ferment arabinose but do not ferment xylose. Dorsey (1963) classified *P. multocida*

Table 2

Comparasion of the acid production from arabinose and xylose by *P. multocida* from the present report and others.

| Investigators | Human | | Swine | | Chicken | | Duck | | Other | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ar | Xy | Ar | Xy | Ar | Xy | Ar | Xy | Ar | Xy |
| Present study | 29 | 65 | 4 | 100 | 82 | 0 | 84 | 11 | - | - |
| Heddleston (1976) | 0 | 91 | 0 | 96 | 13 | 68 | 50 | 75 | 93[a] | 30 |
| Heddleston and Wessman (1975) | 0 | 90 | - | - | - | - | - | - | 5[b] | 75 |
| Oberhofer (1981) | 0 | 71 | - | - | - | - | - | - | - | - |
| Clark *et al* (1984) | 0 | 67 | - | - | - | - | - | - | - | - |
| Heddleston *et al* (1972) | - | - | - | - | 0 | 50 | 0 | 6 | 0[a] | 40 |
| Heddleston (1975) | - | - | - | - | - | - | - | - | −U[c] | +U |
| Dorsey (1963) | - | - | - | - | - | - | - | - | 91[d] | 19 |
| Donahue and Olson (1972) | - | - | - | - | - | - | - | - | 1[e] | 86 |
| Walser and Davis (1975) | - | - | - | - | - | - | - | - | 7[e] | 100 |
| Sakurai *et al* (1986) | - | - | - | - | - | - | - | - | 0[f] | 0 |
| de Alwis and Panangala (1974) | - | - | - | - | - | - | - | - | 19[g] | 59 |
| Shigidi and Mustafa (1979) | - | - | - | - | - | - | - | - | 37[h] | 100 |
| Chandrasekaran *et al* (1981) | - | - | - | - | - | - | - | - | 19[g] | 61 |

Number indicated percent positive; Ar = Arabinose; Xy = Xylose; - = no data;
a = Strain from water fowl; b = Strains from avian and mammals;
c = Strains from avian; −U = Usually negative; +U = Usually positive;
d = Strains from avian; e = Strains from turkeys, f = Strains from green pheasants;
g = Strains from cattle and buffalo; h = Strains from cattle.

isolated from avian origins into 3 groups based on fermentation of xylose, arabinose and dulcitol. Eighty-one percent belonged to group I which did not ferment xylose but arabinose and dulcitol positive. Heddleston (1976) reported that most strains from avian hosts except waterfowl isolates were arabinose negative but xylose positive. Strains from humans exhibited variations in fermentation of arabinose which disagreed the others (Clark *et al,* 1984; Heddleston 1976; Hed dleston and Wessman, 1975; Oberhofer 1981). In general, physical and biochemical characters of *P. multocida* isolated from humans and animals are identical. Our results support the previous reports that specific properties are important in the identification of *P. multocida* but can not be used to determine the host species (Heddleston, 1976; Tanaka, 1926).

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