Pasteurella haemolytica complex of *Pasteurella sensu stricto* as new genus *Mannheimia*: changes in taxonomy

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ABSTRACT: The benefit and usefulness of the new results described by Angen *et al*. (1999) rest upon the necessity for revising the previous taxonomy of *Pasteurellaceae* by grouping microorganisms based on their phylogenetic relationships combined with phenotypic characters and the usefulness of molecular biological methods in determining the taxonomic structure based on genomic evidence with supplementary phenotypic data. According to authors cited the phenotypic character of each serotype, species and even genus was well defined by their morphology, growth requirements and metabolic activities. The genomic relationships of serotypes, species and genera were detected by different molecular biological techniques.

Keywords: *Pasteurella haemolytica*; *Mannheimia*; taxonomy

CONTENTS

1. HISTORY

F. S. Jones (1921) described a microorganism belonging to the *Pasteurella* group but differing mainly in its hemolytic activity. Newsom and Cross (1932) isolated the same microorganism from diseases of calves and sheep, and named it *P. haemolytica*. At the beginning of 1980s Mannheim and co-workers made genetic studies of representative members of the genera *Actinobacillus*, *Haemophilus* and *Pasteurella* (the "AHP group") and they concluded that this group should rank as a family, but some species had been wrongly classified and should be removed from the group. Then Mannheim (1981) proposed the name *Pasteurellaceae* for the family, and considered that the three genera should be retained as distinct entities. In 1981 Pohl suggested that *P. haemolytica* biotype A is more closely linked with *A. lignieresii* than with *Pasteurella*. This supports an earlier suggestion by Mráz (1969a) that *P. haemolytica* should be renamed *A. haemolyticus*. It became clear that the family *Pasteurellaceae* is one of microorganisms which undergoing rearrangements as more microorganisms are being recorded which fall into the group (Biberstein, 1990). Therefor, the taxonomic position within the family should be regarded as changeable (Angen *et al*., 1999).

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2. IDENTIFICATION

2.1. Phenotypic evidence

The relationship of serotypes and biotypes of [*P*.] *haemolytica* was clarified by Biberstein *et al*. (1960) using an indirect haemagglutination test in which he distinguished 11 numbered capsular serotypes (Biberstein and Gills, 1962). The number of recognized serotypes have gradually increased to 17 (Fodor *et al*., 1984, 1987; Younan and Fodor, 1995). Serotypes 3, 4, 10 and 15 were associated with biotype T that of trehalose positive, and the rest serotypes 1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14, 16 and 17 with biotype A that of trehalose negative. Later, nine additional serogroups were demonstrated by counter-current immunoelectrophoresis (Fodor *et al*., 1988). After continuous observation by several investigators, diversifications were designated in obtaining L-arabinose fermentation with some trehalose-negative strains of biotype A (Mráz, 1969a, b; Bisgaard, 1984). Especially, Mráz (1969a, b) signified their differences by series analysis of [*P*.] *haemolytica* in comparison with *Actinobacillus lignieresii* and proposed that it should be classified with *Actinobacillus* rather than with *Pasteurella*. Similar distinctive results were obtained in different laboratories (Bisgaard *et al*., 1986; Mráz, 1977; Mutters *et al*., 1984; Sneath and Stevens, 1985). Prolonged investigations accomplished on bovine and ovine strains, those classified as [*P*.] *haemolytica* or *A. lignieresii*, showed 79 different phenotypic characters and were termed 11 biogroups, which raised the number of biogroups to 12 (Mutters *et al*., 1989). Sneath and Stevens (1990) named *Pasteurella trehalosi* serotypes 3, 4, 10 and 15, which were separated from others by their trehalose positive. And further studies showed the exclusivity of the rest [*P*.] *haemolytica* from the genus *Pasteurella sensu stricto* (Angen *et al*., 1999).

2.2. Genomic evidence

Angen and co-workers clarified those uncertainties for exclusivity of [*P*.] *haemolytica* from *Pasteurella sensu stricto* using methods of molecular biology. Clusters previously identified by ribotyping and multilocus enzyme electrophoresis (MEE) have been evaluated by 16S rRNA sequencing and DNA-DNA hybridizations. The clusters outlined through 16S rRNA analysis were identical to the lineages formerly identified using MEE and ribotyping (Angen *et al*., 1997a, b, c). Furthermore, DNA-DNA hybridizations have confirmed that taxa located in the different clusters (according to ribotyping, MEE or 16S rRNA sequencing) have close genetic affiliation. The overall correspondence noticed between results obtained using ribotyping, multilocus enzyme electrophoresis, 16S rRNA sequencing and DNA-DNA hybridization with phenotypic data was allowed to propose a new genus represented by 5 new species (Angen *et al*., 1999).

3. CURRENT CLASSIFICATION

3.1. Species

Angen and co-workers proposed a new genus *Mannheimia* in the family of *Pasteurellaceae* for the trehalose-negative [*P*.] *haemolytica* complex with its new 5 species based on extensive quantitative evaluation of phenotypic and genomic characteristics (Angen *et al*., 1999). According to the recognized new taxonomy:

New Genus: *Mannheimia*

New Species:

1. *M. haemolytica* (former serotypes 1, 2, 5–9, 12–14 and 16)
2. *M. glucosida* (former biotypes 3A–H and 9, and serotype 11)
3. *M. ruminalis* (former taxon 20 and biotype 3J)
4. *M. granulomatis* (former taxon 18 and biogroup 8D)
5. *M. varigena* (former biogroup 6 and taxa 15, 36)

There are also other strains that are distinct from the aforementioned species and their taxonomic status is not cleared out. They are referred to as *Mannheimia* spp. and included in [*P*.] *haemolytica* biogroups 8A–C, 9, 10 and 12. In addition, there are [*P*.] *haemolytica* like microorganisms that have been isolated from varies species of birds, which are closely related to *Actinobacillus* (Bisgaard, 1977, Angen *et al*., 1999)

3.2. Morphology

Mannheimia consists of Gram-negative, evenly stained short rods (Smith and Phillips, 1990). Most freshly isolated strains are capsulated (Carter, 1967). Gilmour and co-workers (1985) showed that the cells were usually covered with surface protrusions of capsular material.

3.3. Growth requirements

These microorganisms flourished in a casein hydrolysate medium containing 15 individual amino acids, a mixture of salts, vitamins, galactose and glucose (Smith and Phillips, 1990). *Mannheimia* spp. required a higher concentration of iron for production of cytotoxin than was needed for growth. Growth is mesophilic and facultatively anaerobic or microaerophilic (Gentry *et al*., 1987; Angen *et al*., 1999).

3.4. Biochemical activity

All strains of *Mannheimia* ferment mannitol, glucose, maltose, sorbitol, and sucrose are fermented without gas production. Indole, urease, methyl blue (MB) and Voges-Proskauer (VP) reactions are negative. Catalase (almost always) and oxidase are positive (Smith and Phillips, 1990). Typically they do not ferment trehalose, but ferment L-arabinose. *Mannheimia* can be separated from genus *Pasteurella* by not producing acid from D-mannose, from genus *Actinobacillus* (almost) by being urease negative, from genus *Haemophilus* by being mannitol positive and from genus *Lonepinella* by being VP negative (Angen *et al*., 1999).

3.5. Cultural character

Mannheimia grows on blood agar in the form of slight smaller colonies with only a little central thickening. On horse, sheep or rabbit-blood agar forms circular colonies surrounded by a narrow zone of β-haemolysis, but on agar plates made with the blood of very young lambs gives rise to a double-zone of β-haemolysis – an inner complete and an outer wide partial, which increases in size at room temperature. It produces a diffusible substance that enhances the hemolytic effect of *Staphylococcus aureus* β-toxin. *Mannheimia* is very distinct from *Pasteurella* by its growth on MacConkey agar as pink to red colonies (Smith and Phillips, 1990).

4. SOURCE OF VIRULENCE AND PATHOGENICITY

Members of the genus *Mannheimia* produce a number of substances that are associated with the virulence of this group of microorganisms. The most virulent species of this genus is *M. haemolytica*. It is pathogenic for cattle, sheep and goats, but not pathogenic for laboratory animals by the usual methods of infection. Among important substances produced by *M. haemolytica* are the capsule, lipopolysaccharide, iron-regulated outer membrane proteins, toxic outer membrane proteins, adhesins, leukotoxin, and enzymes namely hyaluronidase and neuraminidase (Biberstein and Dwight, 1999). A very useful cellular product in the pathogenesis of diseases caused by *M. haemolytica* is leukotoxin (Burrow *et al*., 1993), which has the ability to lyse erythrocytes of ruminants. This leukotoxin is a 104-kDa protein toxin belonging to the repeats in toxin (RTX) family of toxins, so-called because of the common feature of repeats of glycine-rich sequences within the protein. Interestingly, the hemolysin of *Escherichia coli* is also an RTX-type toxin. Leukotoxin produces a number of biological effects. These include cytotoxic effects upon leukocytes of cattle, sheep and goats (high concentration of Leukotoxin), activation of leukocytes (low concentration Leukotoxin), deaths of leukocytes by apoptosis, and the down regulation of major histocompatibility complex II (MHC II) proteins on the surface of macrophages affecting their ability to present antigen. Activation of macrophages results in the release of the proinflammatory cytokines and interleukin-1 (IL-1), and the stimulation of polymorphonuclear leukocytes leading to the release of H_2O_2, which in turn is converted by alveolar endothelial cells in the presence of Fe^{2+} to hydroxyl radicals. Hydroxyl radicals kill the cell, resulting in accumulation of edema fluid and fibrin (Biberstein and Dwight, 1999; Murphy *et al*., 1995)

5. HABITAT AND DISEASE PATTERNS OF *MANNHEIMIA* SPECIES

All *Mannheimia* spp. can satisfy their exacting growth requirements under the conditions that prevail on the mucous membranes of the upper respiratory tract (URT). In most cases, in competition with other members of normal microflora, these bacteria seem to be able to colonize successfully on the surfaces of URT. Some of these bacteria excrete substances with bactericidal activity against other respiratory tract bacteria (Biberstein and Dwight, 1990). Generally, *M. haemolytica* is known as the most prominent and highly pathogenic microorganism that causes very severe respiratory diseases of ruminants (Biberstein and Dwight, 1999). This is the reason why such so much attention is given to this bacterial species.

5.1. ***Mannheimia haemolytica*** is confined mainly to ruminants, with most strains that have been adequately described originating in cattle, sheep, and goats. Among the most significant *M. haemolytica* infections are the pneumonias of cattle and sheep. The disease often appears in the wake of long hauls by rail or truck or other physiological abuse and is therefore called shipping fever in cattle (Smith and Phillips, 1990; Biberstein and Dwight, 1999).

5.2. ***Mannheimia glucosida*** is mostly isolated from nasal cavity of sheep and causes pneumonia and different disease conditions (Biberstein and Gills, 1962).

5.3. ***Mannheimia ruminalis*** is isolated from rumen of cattle and sheep and its association with disease has not been reported (Bisgaard *et al*., 1986).

5.4. ***Mannheimia granulomatis*** is mostly isolated from rabbits and hares, rarely from cattle and associated with pneumonia, purulent conjunctivitis in leprine species, skin granulomas and other disease conditions in cattle (Ribeiro *et al*., 1989).

5.5. ***Mannheimia varigena*** is isolated from cattle and pigs, and causes pneumonia, sepsis and other diseases (Angen *et al*., 1997b).

Table 1. Disease patterns of new *Mannheimia* spp.

No.	Species	Host animals	Habitat	Etiological significance
1	*M. haemolytica*	cattle	upper respiratory tract	bronchopneumonia septicemia
		sheep	upper respiratory tract	pneumonia septicemia
		goat	upper respiratory tract	pneumonia
2	*M. varigena*	cattle	upper respiratory tract	pneumonia sepsis other disease conditions
		pig	upper respiratory tract	pneumonia sepsis other disease conditions
3	*M. glucosida*	sheep	upper respiratory tract	pneumonia other disease conditions
4	*M. granulomatis*	rabbit and hare	upper respiratory tract	pneumonia purulent conjunctivitis skin granuloma
		cattle		other disease conditions
5	*M. ruminalis*	ruminants	rumen	unknown

For further information see Table 1.

6. ANTIMICROBIAL SUSCEPTIBILITY

The susceptibility of pathogenic species *M. haemolytica* was observed against different antibiotics by agar disc diffusion method (Mevius and Hartman, 2000) and by minimum inhibition concentration (MIC) method using low MIC that is 0.25–1.0 µg/ml of antibiotics (Hormansdorfer and Bauer, 1996). In both tests, *M. haemolytica* isolated from cattle with pneumonia was shown to be resistant (upto 17%) to neomycin, gentamicin, spectinomycin, polymyxin B, flumequine, enrofloxacin, and chloramphenicol, and highly resistant (17 to 80%) to penicillin, amoxicillin, ampicillin, oxacillin, tetracycline, tylosin, trimethoprim-sulphamethoxazole and streptomycin. It was highly sensitive (100%) to ceftiofur and florfenicol (DeRosa *et al.*, 2000; Hormansdorfer and Bauer, 1996; Mevius *et al*., 2000). The above mentioned multiple antibiotic resistance of *M. haemolytica* is increasingly observed as aquired resistance in virtually every pathogenic strain as well as in normal flora. This acquired resistance is carried by plasmids or transposons (Hall *et al*., 1999).

7. CONCLUSION

The question of reclassification of [*P.*] *haemolytica* was raised in the late 1960s. Many investigators showed phylogenetic variation within the genus *Pasteurella sensu stricto* and even at the level of species. The main focus of several analysts was on [*P.*] *haemolytica* complex and their investigations resulted firstly in reclassifying [*P.*] *haemolytica* biotype T as *Pasteurella trehalosi*. And then, Angen and co-workers marked out a taxon, which reclassified the rest [*P.*] *haemolytica* complex to a new genus *Mannheimia* with defined 5 new species. As a result of investigations into this new genus (Angen *et al*., 1999) the following results were obtained. Firstly, phenotypic character of each serotype, species and even genus was well defined based on their morphology, growth requirements and metabolic activities. Secondly, genomic relationships of serotypes, species and genera were detected by different molecular biological techniques.

The benefit and usefulness of the new results rest on the following points:

1. The necessity for revising the previous taxonomy of *Pasteurellaceae* by grouping microorganisms based on their phylogenetic relationships combining with phenotypic characters to its easier and better understanding.

2. The usefulness of molecular biological methods to determine taxonomic structure based on genomic evidences with supplementary phenotypic data.

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