Full Length Research Paper

Phenotypic variability among strains of *Pasteurella multocida* isolated from avian, bovine, caprine, leporine and ovine origin

Sarah O. Ekundayo, Moses O. Odugbo*, Atanda O. Olabode and Philip A. Okewole

National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Phenotypic diversity among 69 field isolates plus 3 vaccine strains previously identified as *Pasteurella multocida* were investigated by extended phenotypic characterization. The field isolates were obtained in Nigeria from chickens (15 isolates), quail (5 isolates), cattle (31 isolates), goats (7 isolates), sheep (8 isolates), rabbits (3 isolates) and the vaccine strains (3 isolates), which are used as prophylaxis against fowl cholera and haemorrhagic septicaemia diseases. Consistent results were obtained for all isolates in the test for Gram reaction, oxidase, catalase, urease, no growth on MacConkey agar and nitrate reduction. All isolates also fermented D-glucose, D-mannitol, and sucrose but failed to ferment lactose. The isolates differed in their ability to ferment L-arabinose, D-dulcitol, D-sorbitol, D-xylose and in the production of indole and H_2S in triple sugar iron agar resulting in the identification of 8 biochemical types or biovars. Dulcitol and sorbitol fermentation patterns meant that the isolates (including the vaccine strains) could be identified as subspecies *Pasteurella multocida multocida* (74%), *Pasteurella multocida septica* (18%), or *Pasteurella multocida gallicida* (8%). The subspecies *P. m. multocida* was demonstrated in all the animal species and the vaccine strains. Among the animal species studied, *P.m. septica* was demonstrated in all but the leporine species while *P.m. gallicida* was demonstrated only in the avian and ovine species. This characterization study adds to the considerable phenotypic variability that has been reported within the *P. multocida* taxon.

Key words: Animal species, Nigeria, *Pasteurella multocida*, phenotypic, subspeciation.

INTRODUCTION

Members of the species *Pasteurella multocida* are ubiquitous and are well-recognized pathogens of many species of animals (Bisgaard, 1993). *P. multocida* causes fowl cholera in poultry , septicaemia and pneumonia in cattle, sheep and goats, and a broad spectrum of conditions ranging from subclincal rhinitis through conjunctivitis, otitis media, pneumonia and septicaemia in rabbits. In Nigeria there are documented reports of pasteurellosis associated with *P. multocida* in poultry (Ambali et al., 2003; Odugbo et al., 2004) and livestock (Ikede, 1977; Akpavie, 1991; Ajuwape et al., 1999).

The *Pasteurella* genus is in a state of nomenclatural flux (Mutters et al., 1985, 1989; Angen et al., 1999). In 1985, Mutters et al. revealed three subspecies within *P. multocida* (*subsp multocida, septica, and gallicida*) using extended phenotypic characterization methods supported by DNA hybridization techniques. These subspecies are often differentiated by fermentation of dulcitol and sorbitol. *P.m. multocida* includes the dulcitol-negative, sorbitol-positive isolates; *P.m. septica* includes the dulcitol-negative, sorbitol-negative isolates; and the *P. m. gallicida* includes the dulcitol- and sorbitol-positive isolates.

In this study we describe the application of Mutter et al. (1985) extended phenotypic tests for the subspeciation of *P. multocida* isolates of avian, bovine, caprine, leporine, and ovine species origin obtained in Nigeria.

*Corresponding author. E-mail: odugm@yahoo.com. Tel: 234-8035956289.

MATERIALS AND METHODS

Bacteria

The isolates of *P. multocida* examined were obtained from various animal species in Nigeria between the years 1960 and 2004. Also included in the study were three *Pasteurella multocida* vaccine strains namely, fowl cholera strain (serotype A:1), haemorrhagic septicaemia standard strain (serotype B:3,4) and haemorrhagic septicaemia African (Obudu) strain (E:2) which are currently employed as prophylaxis for the control of fowl cholera and hae-morrhagic septicaemia in cattle. The field isolates were identified during routine diseases investigation performed on chickens, quail, cattle, sheep, goats, and rabbits at the National Veterinary Research Institute, Vom, Nigeria. All strains had been kept at –70°C in a lyophilized state since the original isolation. Ampoules were cracked open under sterile condition and contents reconstituted in peptone water, which afterwards was inoculated on blood agar and incubated aerobically at 37°C for 24 h.

Phenotypic characterization

The field isolates and the vaccine strains were subjected to a comprehensive phenotypic characterization. Strains were confirmed as *P. multocida* as described by Carter (1990). The isolates were tested for Gram reaction, reaction on triple sugar iron (TSI) agar, motility, production of ornithine decarboxylase, indole, oxidase, catalase and urease, nitrate reduction and inability to grow on MacConkey agar.

The ability to ferment the carbohydrates was tested using a basal medium [1% (w/v) peptone, 0.5% (w/v) NaCl, 0.005% (w/v) bromocresol purple] adjusted to pH 6.8. The basal medium was autoclaved and then aseptically supplemented with sterile carbo-hydrate to a final of 1% (w/v). The carbohydrates tested were: L-arabinose, dulcitol, D-glucose, D-lactose, maltose, D-mannitol, D-sorbitol, D-sucrose, and D-xylose. The complete medium was dispensed in 2 ml volume into sterile bijou bottles. Dense suspen-sions of the organisms (1 loopful in 5 ml peptone water) were prepared and 10 µl added to each carbohydrate bottle. The bottles were incubated at 37°C and read daily for 3 days.

RESULTS

All the field isolates and vaccine strains were identified as *P. multocida* because they were small Gram-negative rods and coccobacilli, were oxidase, catalase and ornithine decarboxylase positive, were urease negative and fermented glucose, mannitol, sucrose but not lactose and showed no visible growth on MacConkey agar, and reduced nitrate to nitrite.

However the field isolates differed in some respect especially in their ability to ferment arabinose, dulcitol, maltose, sorbitol and xylose, and in the production of indole and H_2S gas in TSI agar. These disparities allowed the recognition of 8 biochemical types within the 3 *P. multocida* subspecies (Table 1). The fermentation pattern of *P. multocida* field isolates on dulcitol, sorbitol differen-tiated the 69 field *P. multocida* isolates into 3 subspecies namely, *P. m. multocida* (50 isolates), *P. m. septica* (13 isolates), *P. m. gallicida* (6 isolates); and the vaccine strains (3 isolates) were all of *P.m. multocida* (Table 2).

DISCUSSION

This study has been conducted to provide knowledge on the extended phenotypic properties of *P. multocida* isolates from farmed animal species in Nigeria against the backdrop of current taxonomic knowledge.

We acknowledge few limitations in a study of this type. While our data is limited by absence of reference strains for the 3 subspecies of *P. multocida* (*P.m. multocida, P.m. septica, P.m. gallicida*) our results were however compared with the documented taxonomic reclassify-cation of *P. multocida* as described by Mutters et al. (1989). The retrospective nature of our study meant that there was a dearth of information on the clinical condition associated with the isolates; and serological differen-tiation of the isolates was not undertaken due to lack of antisera to conduct the procedure.

Our biochemical characterization study established 8 biochemical types or biovars within the 3 subspecies of the *P. multocida* taxon. Variable results were found among isolates on fermentation of arabinose, maltose and xylose. Similar variable fermentation pattern of *P. multocida* isolates has also been reported elsewhere (Butt et al., 2003). Other biovars of *P. multocida* have also been reported by previous workers (Bisgaard et al., 1991; Blackall et al., 1997). Of the total 72 field isolates and vaccine strains of *P. multocida* in this study, 74% belonged to the subspecies *multocida* and this sub-species was recorded in all species of animals studied. This is consistent with findings in overseas studies (Fegan et al., 1995; Blackall et al., 1997) where the sub-species *multocida* predominated. The subspecies *septica* was reported in 18% of the total isolates and was present in all but the leporine species of animals studied, while the *gallicida* subspecies accounted for 8% of the total isolates and were present only in the avian and ovine species. All 3 vaccine strains employed as prophylaxis in Nigeria belonged to the subspecies *multocida*.

Worthy of note in our study is the subspecies *gallicida* isolated from fowl cholera outbreak in chickens and ovine pneumonia, in which the 6 strains had two unique bio-chemical characteristics atypical of *P. multocida* - this subspecies isolates were indole negative and produced H_2S on TSI agar (Biotest). Since the studies of Mutters et al. (1985), there have been many studies on the biochem-mical characterization of *P. multocida* subspecies (Mohan et al., 1994; Bisgaard et al., 1991; Fegan et al., 1995; Blackall et al., 1997). To our knowledge while indole negative *P. multocida* isolates may have been reported elsewhere (Madsen et al., 1985; Kamp et al., 1990), variation in the ability of some *P. multocida* isolates to produce H_2S in TSI agar has not been emphasized elsewhere in the literature. Although rare, the isolation of the *gallicida* subspecies has reportedly been implicated in waterfowl and associated birds (Hirsh et al., 1990), in poultry (Fegan et al., 1995) and in pig (Cameron et al.,

Table 1. Some biochemical profiles differentiating the *P. multocida* strains studied.

Test	*P.m. multocida* (Arab+, mal-,xyl-) Biovar 1	*P.m. multocida* (Arab-, mal-, xyl-) Biovar 2	*P.m. multocida* (Arab-, mal+, xyl-) Biovar 3	*P.m. multocida* (Arab+, mal+, xyl-) Biovar 4	*P.m. septica* (Arab- mal+,xyl+) Biovar 5	*P.m. septica* (Arab-, mal-, xyl-) Biovar 6	*P.m. septica* (Arab-, mal+, xyl-) Biovar 7	*P.m. gallicida* (Indol-, H2S[TSI]+) Biovar 8
Indole	+	+	+	+	+	+	+	-
ODC[1]	+	+	+	+	+	+	+	+
H_2S on TSI	-	-	-	-	-	-	-	+
D-sorbitol	+	+	+	+	-	-	-	+
D-dulcitol	-	-	-	-	-	-	-	+
Arabinose	+	-	-	+	-	-	-	+
Maltose	-	-	+	+	+	-	+	+
D-xylose	-	-	-	-	+	-	-	-
Total no. of strains	7	26*	14	6	3	4	6	6

*Including all 3 vaccine strains.
[1]ODC = Ornithine decarboxylase activity.

Table 2. Subspecies of *P. multocida* from various animal species and vaccine strains studied.

Origin	Subspecies of *P. multocida*		
	P.m. multocida	*P.m. septica*	*P.m. gallicida*
Avian	14	1	5
Bovine	23	8	-
Caprine	4	3	-
Leporine	3	-	-
Ovine	6	1	1
Vaccine strains	3	-	-
Total no. of strains	53 (74)*	13 (18)	6 (8)

*Number in bracket is percent isolate.

1996) pasteurellosis. We however suggest that these atypical strains of *gallicida* subspecies encountered in our study be subjected to genotypic characterization (unavailable in our laboratory) for further interpretation.

The implication of the Mutters et al. (1985) sub-speciation of *P. multocida* to the induction of immunity to *P. multocida* in general needs to be investigated. This is against the backdrop that the induction of immunity by *P. multocida* bacteria especially in poultry industry where *P. multocida* plays a prominent role has been reported to be more specific for homologous strains than for other strains (Glisson, 1998). This is even more so that some authors (Biberstein et al., 1990; Holst et al., 1992) have suggested different ecological niches as well as potential differences in pathogenicity for the various *P. multocida* subspecies. For example, the authors observed that *P.m. multocida* and *P.m. septica* are more frequently recovered from "more serious cases of infection"; and

whereas *P.m. multocida* can be isolated from dog- and cat-associated injuries, *P. m. septica* is more frequently isolated from cases with cat contact and may have a greater affinity for the central nervous system. Therefore there may be both epidemiological and clinical Importance to the correct identification of these sub-species.

This characterization study adds to the considerable phenotypic variability that has been reported within the *P. multocida* taxon. From a diagnostician point of view, it is important that one becomes aware of the many variable characteristics of *P. multocida* in order to facilitate its identification. Although the polymerase chain reaction (PCR) test was not conducted in this study, to overcome the difficulties associated with phenotypic characterization, it has been suggested that a PCR test should be included in the criteria used for describing this species. This is in accordance with the report of the ad hoc committee for the re-evaluation of the species definition

in bacteriology (Stackebrandt et al., 2002)

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