

Antibiograms and plasmid profiles of *Pasteurella multocida* isolates from cattle in North Central Nigeria

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Keywords

Cattle – *Pasteurella multocida* –
Plasmid – Resistance to antibiotics –
Nigeria.

Summary

Eighteen strains of *Pasteurella multocida* isolated from zebu cattle at the Veterinary Research Laboratory, National Veterinary Research Institute, Vom, in 2012, were screened for antimicrobial susceptibility and presence of plasmids. Among the 18 isolates studied, 13 (72%) were susceptible to sulfamethoxazole/trimethoprim, 8 (44%) to gentamicin, 8 to amoxicillin / clavulanic acid, 7 (39%) to ciprofloxacin, 7 to chloramphenicol, 5 (28%) to ampicillin, 1 (5.6%) to oxacillin, and 1 to vancomycin. All the isolates were resistant to tetracycline and erythromycin. They all harbored a 5 kb plasmid. Three isolates of serotype E had an additional 3 kb plasmid and one had an additional 6 kb plasmid, but none carried all three plasmids.

■ INTRODUCTION

The Pasteurellaceae family comprises the genera *Actinobacillus*, *Mannheimia*, *Bibersteinia*, *Histophilus* and *Pasteurella*. It is a group of Gram negative, non-motile organisms that includes many pathogenic species for birds, mammals and humans (10). *Pasteurella multocida* is an economically important bacterial pathogen of domestic animals. Hemorrhagic septicemia is an acute septicemic pasteurellosis, caused by *Pasteurella multocida* serotypes B and E, which mainly affects cattle and water buffaloes. The disease occurs in many parts of the world, predominantly in the tropics (4). Plasmid profile analysis is a useful tool in epidemiological studies (12); it has been used in several studies to learn more about the pathogenicity and virulence mechanisms of *P. multocida* (11). Sequence comparisons show that the antibiotic resistance genes found in plasmids exhibit a high degree of sequence homology

to the corresponding genes found in a great variety of gram-negative bacteria (9). Mechanisms of antibiotic resistance in bacteria are varied and include target protection, target substitution, antibiotic detoxification and block of intracellular antibiotic accumulation. Acquisition of genes needed to expand the various mechanisms is greatly aided by a variety of promiscuous gene transfer systems, such as bacterial conjugative plasmids, transposable elements and integron systems that facilitate genes from one deoxyribonucleic acid (DNA) system to another, and from one bacterial cell to another, not necessarily one related to the gene donor (3). Over a period of time *P. multocida* changes its antibiogram and develops resistance to chemotherapeutic agents in use. Therefore, for chemotherapy to be effective, drugs to which the organism has been found sensitive need to be administered during the early phase of the disease (8). This study was conducted to examine the antibiograms and plasmid profiles of *P. multocida* isolated from cattle in North Central Nigeria.

■ MATERIALS AND METHODS

Pasteurella multocida isolates were obtained from the bank of bacteriology division, National Veterinary Research Institute, Vom, Nigeria and had been isolated from the lungs, liver and spleen of zebu cattle that were asymptomatic carriers after necropsy in North Central Nigeria in 2012.

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Antimicrobial susceptibility testing

Susceptibility testing to antibiotics was performed using the standardized single disk diffusion method on Mueller-Hinton agar Schering (New York, USA) (2). Agar was enriched by adding 5% commercially prepared horse serum. The selection of antibiotics was mainly based on the most commonly used antimicrobials against Gram-negative bacteria available in Nigeria and in previous studies such as that by Awar et al. in Pakistan (1). The antimicrobials used were: amoxicillin / clavulanic acid (30 µg), tetracycline (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), oxacillin (5 µg), vancomycin (5 µg), streptomycin (10 µg), ciprofloxacin (5 µg), erythromycin (5 µg), sulfamethoxazole/trimethoprim (25 µg), penicillin (10 IU), and ampicillin (10 µg). All antibiotics were supplied by Oxoid (Basingstoke, UK). Combinations of 12 antibiotic disks were used with no more than six disks per plate. The plates were incubated at 37°C for 24 h. The inhibition zone around each disk was measured independently and compared with standard interpretative charts (6). *P. multocida* vaccine strain B:3.4 was used as control.

Plasmid DNA detection

Isolates were grown overnight at 37°C in 3 ml BHI broth for plasmid isolation. Plasmid DNA was extracted using Plasmid DNA extraction Kit (Thermo Fishers, USA) according to the manufacturer's instructions. The presence of plasmid DNA was detected by agarose gel electrophoresis.

Agarose gel electrophoresis

Plasmid DNA was resolved by electrophoresis in submerged horizontal agarose slab gel (0.9%) in trisborate buffer. The required amount of agarose (Sigma, Aldrich, UK) was dissolved and the DNA ethidium bromide complex gel was visualized on a 320 nm UV transilluminator.

■ RESULTS

The results on the antimicrobial susceptibility of the eighteen isolates of *P. multocida* presented in Table I show a high level of resistance and multiresistance. In particular all isolates were resistant to tetracycline and erythromycin. However, the lowest resistance was observed to sulfamethoxazole/trimethoprim (5/18) and streptomycin (7/18).

The eighteen isolates of *P. multocida* were also examined for the presence of plasmid DNA. All isolates indicated the presence of one or more plasmids. They all harbored a plasmid of about 5 kb molecular weight (Table II). Three isolates had an additional 3 kb plasmid and one had an additional 6 kb plasmid (Figure 1).

■ DISCUSSION

In clinical management of the disease, the antibiotic sensitivity assay serves as a guide to choose the correct antibiotic to be used in the field (5). Bacterial organisms over a period of time change their antibiogram patterns and develop resistance against commonly used chemotherapeutic agents. Amoxicillin is an aminopenicillin-type broad-spectrum antibiotic that acts against many Gram-positive and Gram-negative aerobic and anaerobic bacteria. Amoxicillin is a bactericidal, cell-wall active agent that inhibits bacterial cell-wall synthesis by binding to penicillin-binding proteins and inhibits cross-linking of bacterial peptidoglycan. Clavulanic acid is a beta-lactam antibiotic, structurally related to penicillin. It possesses the ability to inactivate a wide range of

beta-lactamase enzymes commonly found in microorganisms resistant to penicillin and cephalosporin. In this study, the number of strains resistant to amoxicillin / clavulanic acid (10, i.e. 55.5%) was lower than that resistant to penicillin (17, i.e. 94.4%).

Table I

Antibiotic resistance of *Pasteurella multocida* isolated from cattle

Antimicrobial agent	Num. (%) of resistant strains (n = 18)
SXT	5 (27.8)
S	7 (38.9)
AMC	10 (55.5)
CN	10 (55.5)
CIP	11 (61.1)
C	11 (61.1)
AMP	13 (72.2)
P	17 (94.4)
OX	17 (94.4)
VA	17 (94.4)
TE	18 (100)
E	18 (100)

SXT = sulfamethoxazole/trimethoprim; S = streptomycin; AMC = amoxicillin / clavulanic acid; CN = gentamicin; CIP = ciprofloxacin; C = chloramphenicol; AMP = ampicillin; P = penicillin; OX = oxacillin; VA = vancomycin; TE = tetracycline; E = erythromycin.

Table II

Plasmid profiles of 18 *Pasteurella multocida* strains isolated from cattle

Num.	<i>P. multocida</i> isolates	Estimated size of harbored plasmids		
		6 kb	5 kb	3 kb
1	Ka2	-	+	+
2	Ot2	-	+	-
3	JN18	-	+	-
4	Mg7	-	+	+
5	Mg4	-	+	-
6	JN6	-	+	-
7	JN14	-	+	-
8	Bld10	-	+	-
9	Bld9	-	+	-
10	Bld3	-	+	-
11	JST2	-	+	-
12	JN5	-	+	-
13	Ka5	-	+	-
14	Ka3	+	+	-
15	Jst8	-	+	-
16	JN12	-	+	+
17	Ka4	-	+	-
18	JN3	-	+	-

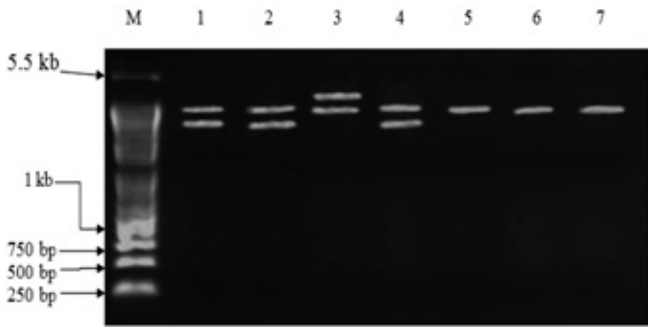


Figure 1: Observed plasmid profiles of *Pasteurella multocida* isolates from cattle. Lane M: 1 kb DNA molecular weight marker (Invitrogen®); Lane 1: Ka2 isolate; Lane 2: Ot2 isolate; Lane 3: Ka3 isolate; Lane 4: Mg7 isolate; Lane 5: Mg4 isolate; Lane 6: Jn6 isolate; Lane 7: Jn14 isolate. Band sizes of 6.0 kb, 5.5 kb and 3.0 kb plasmids were isolated.

■ CONCLUSION

All isolates harbored a similar plasmid of 5 kb. Three isolates from serotype E had an additional 3 kb plasmid and one isolate had an additional 6 kb plasmid, but none of the isolates carried all three plasmids. All the isolates were resistant to tetracycline and erythromycin. However, the lower resistance of the isolates to sulfamethoxazole/trimethoprim and streptomycin may suggest that they are more effective and may be used for the treatment of *P. multocida*, the agent involved in hemorrhagic septicemia. In Nigeria the use of antimicrobial drugs in animals is not regulated. Therefore farmers can purchase them without prescriptions. The indiscriminate use of antimicrobials by farmers may be responsible for the high levels of multiple resistances. There is a need to educate cattle owners on the dangers of indiscriminate use of drugs.

Acknowledgments

The authors wish to acknowledge the management of the National Veterinary Research Institute, Vom, Nigeria, for supporting this research and for permission to publish this work.

Résumé

Sugun M.Y., Kwaga J.K.P., Kazeem H.M., Ibrahim N.D.G. Anti-biogrammes et profils plasmidiques d’isolats de *Pasteurella multocida* de bovins dans le centre nord du Nigeria

Des essais sur la sensibilité d’antimicrobiens et la présence de plasmides ont été effectués sur dix-huit souches de *Pasteurella multocida* isolées à partir de zébus du Laboratoire de recherche vétérinaire, Institut national de recherche vétérinaire, Vom, en 2012. Parmi les 18 isolats, 13 (72 p. 100) étaient sensibles à la sulfaméthoxazole/triméthoprime, 8 (44 p. 100) à la gentamicine, 8 à l’acide amoxicilline / acide clavulanique, 7 (39 p. 100) à la ciprofloxacine, 7 au chloramphenicol, 5 (28 p. 100) à l’ampicilline, 1 (5,6 p. 100) à l’oxacilline, et 1 à la vancomycine. Tous les isolats étaient résistants à la tétracycline et à l’érythromycine. Tous hébergeaient un plasmide de 5 kb. Trois isolats du sérotype E ont présenté un plasmide supplémentaire de 3 kb, un autre a exhibé un plasmide supplémentaire de 6 kb mais aucun des isolats ne contenait à la fois les trois plasmides.

Mots-clés : Bovin – *Pasteurella multocida* – Plasmide – Résistance aux antibiotiques – Nigeria.

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Accepté le 07.04.2014

Resumen

Sugun M.Y., Kwaga J.K.P., Kazeem H.M., Ibrahim N.D.G. Anti-biogramas y perfiles de plásmidos de *Pasteurella multocida* aislados en ganado en el norte central de Nigeria

Se aislaron dieciocho cepas de *Pasteurella multocida* en ganado cebú en el Laboratorio de investigación Veterinaria, Instituto Nacional de Investigación Veterinaria, Vom, en 2012, y se estudió la susceptibilidad antimicrobiana y la presencia de plásmidos. De los 18 aislamientos estudiados, 13 (72%) fueron susceptibles a sulfametazona/trimetoprim, 8 (44%) a gentamicina, 8 amoxicilina/ ácido clavulánico 7 (39%) a ciprofloxacina, 7 a cloranfenicol, 5 (28%) a ampicilina, 1 (5,6) a oxaciclina y 1 a vancomicina. Todos los aislamientos fueron resistentes a tetraciclina y eritromicina. Todos portaron el plásmido 5 kb. Tres aislamientos del serotipo E tuvieron adicionalmente el plásmido 3 kb y uno tuvo el plásmido 6 kb adicional, pero ninguno fue portador de los tres plásmidos.

Palabras clave: Ganado bovino – *Pasteurella multocida* – Plásmido – Resistencia a los antibióticos – Nigeria.

