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RESEARCH ARTICLE

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

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Abstract

A total of 18 *P. multocida* isolated in (2012) from zebu cattle at the Veterinary Research Laboratory, National Veterinary Research Institute Vom were screened for their antimicrobial susceptibility and the presence of plasmids. Among the 18 isolates studied, 13(72.2) were susceptible to sulphamethoxazole/trimethoprim, 8(44.4) susceptible to gentamicin and amoxycillin/clavulanic acid, 7(38.9) were susceptible to Ciprofloxacin and chloramphenicol, 1(5.6) were susceptible to oxacillin and vancomycin each and 5(27.8) to ampicillin. All the isolates 18(100%) were resistant to tetracycline and erythromycin. All the isolates harboured a 5kb plasmids. Three of the serogroup E isolates had a 3kb additional plasmid, and one isolate had a 6kb additional plasmid; but none of the isolates carried all 3 plasmids.

Introduction

The Pasteurellaceae family comprises the genera Actinobacillus, Mannhemia, Bibersteinia, Histophilus and Pasteurella. It is a group of Gram negative, nonmotile organisms that includes many species pathogenic for birds, animal and man (11). *Pasteurella multocida* is an economically important bacterial pathogen of domestic animals. Haemorrhagic septicaemia (HS) is an acute septicaemic pasteurellosis, caused by *Pasteurella multocida* serotype 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 (15). Plasmid has been used in several studies in order to learn more about the pathogenicity and virulence mechanisms of *P. multocida* (14). Sequence comparisons showed that the antibiotic resistance genes found in plasmids exhibited 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* changed its antibiogram and developed resistance to chemotherapeutic agents in use. Therefore for chemotherapy to be effective drug to which the organism has been found sensitive need to be administered during the early phase of the disease before specific clinical signs appear (Gupta et al., 1996). This study was conducted to examine the antibiograms and plasmid profiles of *Pasteurella multocida isolated* from cattle in North Central Nigeria.

MATERIALS AND METHODS

The isolates, *Pasteurella multocida* were obtained from the isolate bank of bacteriology division, National Veterinary Research Institute Vom, Nigeria and had been isolated from (lungs, liver and spleen) of zebu cattle that were asymptomatic carriers after necropsy in the north central Nigeria in 2012.

Antimicrobial susceptibility testing.

Susceptibility testing was performed using the standardized single disk diffusion method on Mueller-Hinton agar (New York, USA) (2). Agar was enriched by the addition of 5 % commercially prepared horse-serum. The selection of antibiotics was based largely on the most commonly used antimicrobial against Gram negative bacteria available in Nigeria and previous studies e.g., antibiotic sensitivity of *Pasteurella multocida* isolated from cattle and buffaloes in Pakistant (1).The antimicrobial used are: Amoxycillin/clavulanic acid AMC (30 µg), TE (5 µg), Chloramphenicol C (30 µg), Gentamicin CN (10 µg), Oxacillin OX (5 µg), Vancomycin VA(5 µg), Streptomycin S (10µg), Ciprofloxacin CIP (5 µg), Erythromycin E (5 µg), Sulphamethoxazole/trimethoprim SXT (25 µg), Penicillin P (10 i/u), Ampicillin AMP (10 µg). All antibiotics were supplied by Oxoid (Basingstoke, UK). Combinations of 12 antibiotic disks were used with no more than 6 disks per plate. Then the plates were incubated at 37 ^{0}C for 24 h. The inhibition zone around each disk was measured independently and compared with standard interpretative charts: (Clinical Laboratory Standards Institute) (6). Zone size for each antimicrobial agent was measured independently before comparison. *Pasteurella multocida* vaccine strain B: 3.4 was used as control.

Plasmid DNA detection

Isolates were grown overnight at 37 ^{o}C in 3 mL BHI broth for plasmid isolation. Plasmid DNA was extracted using Plasmid DNA extraction Kit (Thermo Fishers NY,USA), according to 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 tris-borate buffer (TBE). 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

Eighteen isolates of *P. multocida* were examined for antimicrobial susceptibility. The results showed a high level of resistance and multiresistance among the tested isolates. The isolates were all resistant to tetracycline and erythromycin. Among the 18 isolates tested, 13 were susceptible to sulphamethoxazole/trimethoprim, and 11 of isolates were susceptible to streptomycin. Only 8 of isolates were susceptible to amoxycillin/clavulanic acid and to gentamicin, 7 were susceptible to ciprofloxacin and chloramphenicol. Susceptibility to ampicillin was found in 5 of isolates, while only one isolate was susceptible to penicillin, oxacillin and vancomycin (Table I).

The eighteen isolates of *P. multocida* were also examined for the presence of plasmid DNA. All isolates indicated presence of one or more plasmid. All isolates of *P. multocida* tested harboured a plasmid of about 5kb molecular weight. Three isolates had an additional 3kb plasmid and one isolate named "Ka3" harboured an additional 6kb plamid. (Table II and Figure I).

Figure I: Observed plasmid profiles of *P. multocida* isolates from cattle.

Lane M – 1kb DNA molecular weight marker (*Invitrogen®)*. Lane 1- Ka2 isolate, lane2- Ot2 isolate, lane3- Ka3 isolate, lane4- Mg7 isolate, lane 5- Mg4 isolate, lane 6-Jn6 isolate, lane7- Jn14 isolate. The band sizes of 6.0kb, 5.5kb and 3.0kb plasmids were isolated.

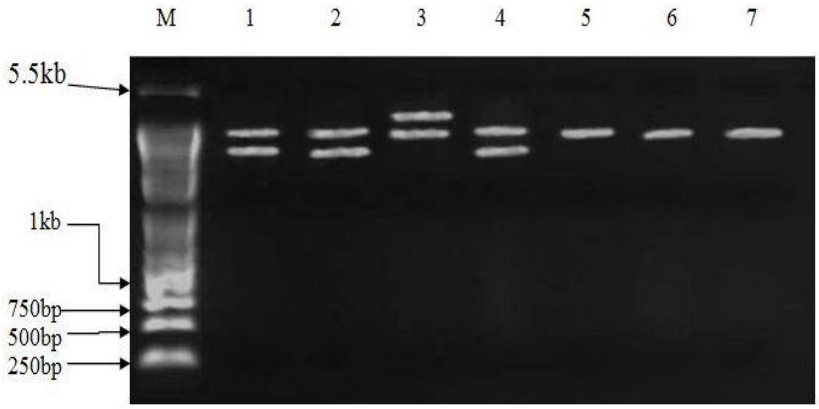

Table II : Plasmid profiles of 18 *P. multocida* isolated from Cattle.

S/No.	*P. multocida* isolates	Estimated sizes of harbored plasmids		
		6 kb	5 kb	3 kb
1	Ka2	_	+	+
2	Q2	_	+	-
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	_	+	_

Table I: Antimicrobial susceptibility patterns of 18 *Pasteurella multocida* isolated from cattle

Antimicrobial agent	No. (%) of strains resistant n=18	No. (%) of strains sensitive n=18
SXT	5(27.8)	13(72.2)
S	7(38.9)	11(61.1)
AMC	10(55.5)	8(44.4)
CN	10(55.5)	8(44.4)
CIP	11(61.1)	7(38.9)
C	11(61.1)	7(38.9)
AMP	13(72.2)	5(27.8)
P	17(94.4)	1(5.6)
OX	17(94.4)	1(5.6)
VA	17(94.4)	1(5.6)
TE	18(100)	0(0.0)
E	18(100)	0(0.0)

AMC=Amoxycillin/clavulanic acid, TE=Tetracycline, C=Chloramphenicol, CN=Gentamicin, OX=Oxacillin, VA=Vancomycin, S=Streptomycin, CIP=Ciprofloxacin, E=Erythromycin, SXT=Sulphamethoxazole/trimethoprim, P=Penicillin, AMP = Ampicillin.

DISCUSSION

In clinical management of the disease, 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 possesses activity 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 the cross-linking of bacterial peptidoglycan. Clavulanic acid is a beta-lactam, structurally related to the penicillins. It possesses the ability to inactivate a wide range of beta-lactamase enzymes commonly found in micro-organisms resistant to penicillins and cephalosporins. In this study number of strains resistant to Amoxycillin/clavulanic acid (AMC) 10(55.5%) not higher than to Penicillin (P) 17(94.4).

Conclusion

All isolates harboured a similar plasmid of 5kb. Three isolates from group E had an additional 3kb plasmid and one isolate "(ka3") had a 6kb plasmid; but none of the isolates carried all 3 plasmids. Among the 18 isolates studied, 10 were sensitive to sulphamethoxazole/trimethoprim, 10 susceptible to gentamicin and amoxycillin/clavulanic. Ciprofloxacin 7, chloramphenicol 7, oxacillin, penicillin and vancomycin 1 each, and ampicillin 5. All the isolates 18 were resistant to tetracycline and erythromycin. Also the susceptibility of the isolates to Sulphamethoxazole/trimethoprim and streptomycin having less resistance among the isolates tested in this study may be suggestive of fact that they are more effective and may be used for the treatment of *P. multocida* causative agent of HS. Antimicrobial use in animal in Nigeria is not regulated; farmers are able to purchase these off-the shelf without prescription. This results in the indiscriminate use of antimicrobial by farmers and may be responsible for the high levels of multiple resistance. There is a need to educate the cattle owners on the dangers of indiscriminate use of drugs.

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