Nigerian Veterinary Journal
Vol 33 (4) 617-623

Antimicrobial Resistance in *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Isolated from the Milk of Dairy Cows in Three Nigerian Cities

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SUMMARY

Bovine mastitis is usually associated with bacterial pathogens isolated from the milk or mammary glands of dairy cows. A total of 205 isolates comprising of 110 (53.66%) Escherichia coli, 67 (32.68%) Klebsiella pneumoniae, and 28 (13.66%) Pseudomonas aeruginosa obtained from cases of bovine mastitis from southwest and Northern Nigeria during a period of one year were tested for susceptibility to ampicillin, tetracycline, neomycin, streptomycin, sulphadimidine and nalidixic acid using minimum inhibitory concentration (MIC) method. The results demonstrated wide variation of in the susceptibility patterns for the various organisms from different regions of Nigeria. The three organisms displayed highest resistance to sulphadimidine 200 (97.5%) followed by ampicillin 153 (74.63%), tetracycline 103 (50.24%), neomycin 90 (43.90%), streptomycin 68 (33.17%) and nalidixic acid 29 (14.15%) respectively. The resistance patterns of the strains revealed 27 distinct resistance groups. In conclusion these data confirmed that majority of the Gram negative organisms that are causative agents of mastitis in Nigeria were resistant to several antibiotics. This could be a result of indiscriminate use of drugs by dairy farmers for treatment of mastitis in their herds.

INTRODUCTION

About 135 different microorganisms have been isolated from bovine intramammary infections (IMIs) and the contribution of various species of bacteria to mastitis has shifted over time with the majority of IMIs caused by Gram-negative bacteria (Bradley, 2002). Mastitis is one of the major indications of antibiotic use in dairy cows (DANMAP, 2002). Measures to control mastitis such as improved milking hygiene have reduced the prevalence of major pathogens such as Streptococcus agalactiae (Pol and Ruegg, 2007; Pitkala et al., 2004). The use of antimicrobial agents in the treatment of infectious diseases has produced great advances in human medicine as well as in veterinary medicine. The efficacy of antimicrobial agents on pathogenic organisms has resulted in better control of infectious diseases, great economic benefits to livestock owners and increased animal protein to combat malnutrition in many parts of the world. However, the extensive use of antimicrobial agents has resulted in the emergence of resistant strains of bacteria some of which harbour R-factor (Aryal, 2001).

Resistance of mastitis pathogens to antimicrobial agents is a well-documented challenge in dairy cows (Owen et al., 1997; Rossitto et al., 2002; Erskine et al., 2002; Pol and Ruegg, 2007; Pitkala et al., 2004). The World Health Organization (WHO) stated that antimicrobial use is associated with the risk of transfer of resistant pathogens between man and animals (WHO, 1998; WHO, 2002). Hence, there is need for regular investigation and monitoring of the use of antimicrobial agents in food-producing animals as well as the prevalence of resistant mastitis pathogens (Rossitto et al., 2002; Osteras et al., 2005; Aarestrup, 2005).

Resistance to antimicrobial agents in mastitis pathogens has two relevant aspects; the first is a reduction in cure rates after treatment of clinical mastitis cases (Owen et al., 1997; Sol et al., 2000). The second issue is the potential impact of transmission of resistant bacteria to human beings via the food chain (Murray, 2004). Direct transmission of pathogens to humans from milk of animals with clinical mastitis may not likely to occur where such milk is banned for human consumption. However, in cases of subclinical or latent infections, resistant bacteria present in the bulk milk tank may be transmitted to humans via raw milk products (Amosun et al., 2009). Antibiotic therapy of lactating cows also predisposes to the risk of antibiotic residues in meat or milk through errors in record keeping and by variation among cows in antibiotic excretion rate. Intramammary treatment is associated with the additional the additional risk of introducing pathogens into the treated quarter through lapses in hygiene such as dirty teat ends. Treatment may also reduce host defense by damaging the keratin plug in the teat canal (Nickerson, 1987). Using antibiotics to treat clinical mastitis is only rational if the benefits outweigh the cost of medications, discarded milk, and residue risk (Gutterbock et al., 1993).

This investigation was carried out to determine the incidence of antimicrobial resistance in some Gram negative bacteria isolated from milk samples obtained from dairy cows in herds with cases of bovine mastitis.

MATERIALS AND METHOD

A total of 110 Escherichia coli, 67 Klebsiella pneumoniae and 28 Pseudomonas aeruginosa pure isolates from milk randomly obtained from dairy herds across the south, middle belt and northern Nigeria. These Gram negative organisms were studied for antibiotic susceptibility to ampicillin, tetracycline,

TABLE I: Distribution of antimicrobial resistant Gram-negative bacterial isolates from milk of dairy cows in three Nigerian cities

Antimicrobial	% resistant *E. coli*			% resistant *Klebsiella pneumoniae*			% resistant *Pseudomonas aeruginosa*		
	Ibadan (n=28)	Ilorin (n=39)	Kaduna (n=43)	Ibadan (n=31)	Ilorin (n=19)	Kaduna (n= 17)	Ibadan n=19	Ilorin (n= 9)	Kaduna (n= 0)
Ampicillin	85.7	100	18	100	79	94.1	84.2	44.4	0
Tetracycline	50	74.4	65.1	19.4	31.6	17.7	73.7	33.3	0
Streptomycin	53.6	2.6	58.1	51.6	5.3	0	52.63	0	0
Sulphadimidine	100	100	100	87.1	94.7	100	100	100	0
Neomycin	21.4	25.6	48.8	96.8	57.8	29.4	36.8	0	0
Nalidixic acid	28.6	35.9	0	19.4	0	5.9	0	0	0

sulphadimidine, streptomycin, neomycin and nalidixic acid (sourced from Sigma Aldrich Chemical Ltd, USA).

The minimum inhibition concentrations (MIC) of 6 antibiotics namely ampicillin, tetracycline, sulphadimidine, streptomycin, neomycin and nalidixic acid for each of the Gram negative isolates was determine respectively by tubes method as previously described by Rollins et al., (2003). The susceptibilities of the isolates to the various antimicrobials were determined using their MICs and recorded as either sensisitive or resistant according to the Clinical Laboratory Standards Institute (CLSI).

RESULTS

The antibiotic resistance among the E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa obtained from the cows varied as shown in Table I. From Ibadan, all 28 (100%) E. coli isolates were resistant to sulphadimidine while 24 (85.7%), 15 (53.6%), 14 (50.0%), 8 (28.6%) and 6 (21.4%) were resistant to ampicillin, streptomycin, tetracycline, nalidixic acid and neomycin respective. All the 31 (100%) Klebsiella pneumoniae isolates were resistant to ampicillin while 30 (96.77%), 27 (87.10%), 16 (51.61%), 6 (19.35%) and 6 (19.35%) were resistant to neomycin, sulphadimidine, streptomycin, tetracycline and nalidixic acid

Table II: Total number of *E.coli, Klebsiella pneumoniae and pseudomonas aeruginosa* isolated showing resistance to Antibiotics.

Antimicrobial agent	Number of bacterial isolates resistance (%)
Sulphadimidine	200(97.6%)
Ampicillin	153(74.6%)
Tetracycline	103(50.2%)
Neomycin	90(43.9%)
Streptomycin	68(33.2%)
Nalidixic acid	29(14.2%)

TABLE III: Antimicrobial resistance patterns of Gram negative bacterial isolates from milk of dairy cows in three Nigerian cities

Resistance Pattern	*Escherichia coli (110)*	*Klebsiella pneumonia (67)*	*Pseudomonas aeruginosa (28)*
Su	3	1	3
PN	0	1	0
PNSu	11	11	0
TeSu	5	2	3
PNN	0	1	0
SuN	2	2	0
SSu	1	0	1
PNTeSu	25	4	7
PNSuN	2	19	1
TeSuN	3	0	0
TeSSu	8	0	0
PNSSu	3	0	2
PNSuNa	2	1	0
PNSN	0	3	0
SSuN	10	0	1
PNSSuNa	4	0	0
PNTeSSu	2	0	2
PNTeSuN	4	5	1
PNTeSuNa	6	1	4
TeSSuN	7	0	0
PNSuNNa	1	1	0
PNSSuN	0	9	0
PNTeSuSN	2	2	3
PNTeSSuNa	3	0	0
PNTeSuNNa	5	1	0
PNSSuNNa	0	3	0
PNTeSSuNNa	1	0	0

respectively. Moreover, all 19 (100%) Pseudomonas aeruginosa isolates were resistant to sulphadimidine while 16 (84.21%), 14 (73.68%), 10 (52.63%) and 7 (36.84%) were resistant to ampicillin, tetracycline, streptomycin and neomycin respectively. The Pseudomonas aeruginosa isolates all were sensitive to nalidixic acid

Table IV: Summary of the resistance pattern for bacterial isolates.

Resistance patterns	*Escherichia coli* (110)	*Klebsiella pneumoniae* .(67)	*Pseudomonas aeruginosa* (28)
Mono resistance	3	2	3
Double resistance	19	16	4
Triple resistance	53	27	11
Quadruple resistance	24	16	7
Quintuple resistance	10	6	3
Sextuple resistance	1	0	0

In Ilorin, all 39 (100%) E. coli isolates were resistant to sulphadimidine and ampicillin while 29 (74.36%), 14 (35.90%), 10 (25.64%) and 1 (2.56%) showed resistance to tetracycline, nalidixic acid, neomycin and streptomycin respectively. For Klebsiella pneumonia, 18 (94.74%), 15 (78.95%), 11 (57.89%), 6 (31.58%), 1 (5.26%) and 0 (0%) isolates were resistant to sulphadimidine, ampicillin, neomycin, tetracycline, streptomycin and nalidixic acid respectively. Meanwhile, all 9 (100%) Pseudomonas aeruginosa isolates were resistant to sulphadimidine while 4 (44.44%) and 3 (33.33%) isolates showed resistance to ampicillin and tetracycline respectively. However, the isolates were all sensitive to nalidixic acid, neomycin and streptomycin.

In Kaduna, all 43 (100%) E. coli isolates were resistant to sulphadimidine while 28 (65.12%), 25 (58.14%), 21 (48.84%), 8 (18.60%) and 0 (0%) were resistant to tetracycline, streptomycin, neomycin, ampicillin, and nalidixic acid respectively. For Klebsiella pneumonia, 17 (100%), 16 (94.12%), 5 (29.41%), 3 (17.65%), 1 (5.88%) and 0 (0%) isolates were resistant to sulphadimidine, ampicillin, neomycin, tetracycline, nalidixic acid and streptomycin respectively.

The overall resistance displayed by all the isolates against the tested antibiotic were highest for sulphadimidine (97.5%) followed by ampicillin (74.63%), tetracycline (50.24%), Neomycin (43.90%), streptomycin (33.17%) and Nalidixic acid (14.15%) respectively as shown in table II. The resistance patterns exhibited by all the strains revealed 27 distinct resistance groups (Table III). These resistance patterns can be categorized as mono-resistance (3.9%), double resistance (19.02%), triple resistance (44.39%), quadruple resistance (22.93%), quintuple resistance (9.27%) and sextuple resistance (0.49%) (Table IV).

DISCUSSION

The use of antibiotics in food animal production has been a great public health and food safety concern. Although these drugs affordable and enhance the productivity of food animal industry but their use results in the spread of resistant bacteria and the associated public health hazards (Langlios and Dawson, 1999).

In this investigation, the fresh milk samples contained 53.66% Escherichia coli, 32.68% Klebsiella pneumoniae and 13.66% Pseudomonas aeruginosa isolates. It was observed that all of these isolates were resistant to one or more commonly used antimicrobials tested in this study. This study showed that Escherichia coli isolated from Ibadan, Ilorin and Kaduna demonstrated resistance tetracycline, ampicilin and sulpha drugs. Multiple antibiotics resistance was also observed among the isolates. The findings in this study showed that Pseudomonas aeruginosa isolates were highly resistance to ampicillin this finding is similar to what was reported by Watt et al., (1995). Ampicillin resistance in E. coli is always due to beta-lactamase production (Arias and Murray, 2009). Claves fed with such milk are also at risk of bacterial enteritis and diarrhoea

There has been global concern on the possible development of resistance to antimicrobial agents in organisms studied as a result of misuse of drugs without carrying out appropriate sensitivity test (Aarestrup, 2005). Antibiotics are administered by herdsmen to cattle on routine basis as prophylactic agents as well as chemotherapeutic agents. This has chiefly contributed to increasing prevalence of resistance food-borne pathogens since past decades (Threlfall et al., 2000; Chiu et al., 2002). Selection pressure for resistance is created by indiscriminate use of antimicrobials in food-producing animals (Bywater, 2004). The contamination of by resistant pathogens obtained in this study is of public health significance because the resistance factors may be transmitted to human population. Adetosoye, (1980) showed that Escherichia coli strains can harbour R-plasmids which are transmissible to sensitive recipients such as Escherichia coli K12 or to Pseudomonas aeruginosa and among enteric commensal and pathogenic bacteria. This may lead to antimicrobials treatment complications and further propagation of resistance.

CONCLUSION

This work confirmed high prevalence of antimicrobial resistance among Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa which are causative agents of mastitis and can contaminate fresh milk, thereby rendering such milk unsafe for consumption. However, the local herdsmen and milkmaids consumed that raw unpasteurised milk are also at risk of milk borne zoonoses by these pathogens.

The high prevalence of antimicrobial resistance could have resulted from misuse of these drugs by dairy farmers for treatment of mastitis in their herds. Therefore, indiscriminate use of antibiotics and consumption of fresh milk should be discouraged. Also responsible therapeutic use of antibiotics against mastitis should follow appropriate diagnosis and antibiotic susceptibility tests.

REFERENCES

AARESTRUP, F.M. (2005): Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin. Pharmacol. Toxicol. 96: 271-281.

ADETOSOYE, A.I. (1980): Transmissible drug resistance in human and animal strains of Enterobacteriaceae and Pseudomonas aeruginosa. Res. Vet. Sci. 29: 342-345.

AMOSUN, E.A, OLATOYE, I.O. and ADETOSOYE, A.I. (2009): The prevalence of gram negative bacteria in bovine mastitis among dairy herds in Nigeria. Trop. Vet. 27(3): 25-29.

ARIAS, C.A. and MURRAY, B.E. (2009): "Antibiotic-Resistant Bugs in the 21st Century — A Clinical Super-Challenge". New Engl. J. Med. 360 (5): 439–443.

ARYAL, S. (2001). Antibiotic Resistance: A Concern to Veterinary and Human Medicine. Nepal Agric. Res. J. 4 & 5: 66-70.

BRADLEY, A.J. (2002). Bovine mastitis: An evolving disease. Vet. J. 164:116-128.

BYWATER, R. J. (2004): Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. J. Vet. Med. B 51:361–363.

CHIU, C.H., WU, T.L., SU, L.H., CHU, C., CHIA, J.H., KUO, A.J., CHIEN, M.S. and LIN, T.Y. (2002): The emergence in Taiwan of fluoroquinolone resistance in Salmonella enterica serotype Choleraesuis. N. Engl. J. Med 346:413–419.

DANMAP, (2002): Use of antimicrobial agents and occurrence of antimicrobial agents and resistance in bacteria from food animals, foods and humans in Denmark. Available at: http://www.danmap.org/pdffilesdanmap.2002.pdf

ERSKINE, R.J., WALKER, R.D., BOLIN, C.A., BARTLETT, P.C. and WHITE, D.G. (2002): Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. J. Dairy Sci. 85:1111-1118.

GUTTERBOCK, W.M., VAN EENENNAAM, A.L., ANDERSON, R.J., GARDNER, I.A., CULLOR, J.S. and HOLMBERG, C.A. (1993): Efficacy of intramammary antibiotic therapy for treatment of clinical mastitis caused by environmental pathogens. J. Dairy Sci. 76:3437-3444.

LANGLOIS B.E. and DAWSON, K.A. (1999): Antimicrobial resistance of Gram-negative enteric bacteria from pigs in a non-antimicrobial exposed herb before and after transportation. J. Food Protect. 62: 797-799.

MURRAY, A.C., KUSKOWSKI, M.A. and JOHNSON, J.R. (2004): Virulence factors predict Escherichia coli colonization patterns among human and animal house hold members. Ann. Intern. Med. 140:848-849.

NICKERSON, S.C. (1987): Resistance mechanisms of the bovine udder: new implications for mastitis control at the teat end. J. Am. Vet. Med. Assoc. 191:1484.

OSTERAS, O., HOGEVEEN, H., SINGH, D.K. and LESLIE, K.E. (2005): Economic consequences of mastitis. IDF Bulletin 394/2005. International Dairy Federation, Bruxelles, Belgium.

OWENS, W.E., RAY, C.H., WATTS, J.L. and YANCEY, R.J., JR. (1997): Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. J. Dairy Sci. 80:313-317.

PITKALA, A., HAVERI, M., PYORALA, S., MYLLYS, V. and HONKANEN-BUZALSKI, T. (2004): Bovine mastitis in Finland 2001 – Prevalence, distribution of bacteria, and antimicrobial resistance. J. Dairy Sci. 87:2433-2441.

POL, M. and RUEGG, P.L. (2007): Relationship between antimicrobial drug usage and antimicrobial susceptibility of gram-positive mastitis pathogens. J. Dairy Sci. 90:262-273.

ROLLLINS, D.M., TEMENAK, J.J, SHIELD, P. and JOSEPH, S.W. (2003): Microbial Pathogeesis Laboratory Manual. 2nd Edition Maryland Univ. 156p.

ROSSITTO, P.V., RUIZ, L., KIKUCHI, Y., GLENN, K., LUZ, K., WATTS, J.L. and CULLOR, J.C. (2002): Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in Central California dairies. J. Dairy Sci. 85:132-138.

SOL, J., SAMPIMON, O.C., BARKENA, H.W. and SCHUKKEN, Y.H. (2000): Factors associated with cure after therapy of clinical mastitis caused by Staphylococcus aureus J. Dairy Sci. 83:278-284.

THRELFALL, E. J., WARD L.R., FROST, J.A. and WILLSHAW, G.A. (2000): The emergence and spread of antibiotic resistance in food-borne bacteria. Int J Food Microbiol 62:1–5.

WATTS, J.L., SALMON, R.J., YANCEY JR., S.C. NICKERSON, S.C., WEAVER, J., HOLMBERG, C., PANKEY, J.W. and FOX, L.K. (1995): Antimicrobial susceptibility of microorganisms isolated from the mammary glands of dairy heifers. J. Dairy Sci. 78: 1637-1648.

WHO (World Health Organisation) (1998): Zoonotic non O157 Shiga toxin-producing E. coli (STEC). Report of a WHO scientific working group meeting WHO/CSR/APH/98.8 http://www.who.int/emc.

WHO (2002). Use of antimicrobials outside human medicine and resultant antimicrobial resistance in humans. http://www.who.int/emc/diseases/zoo/who_global_principles/index.htm accessed on June 17, 2012.