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SENSITIVITY OF *LISTERIA* SPECIES, RECOVERED FROM INDIGENOUS CHICKENS TO ANTIBIOTICS AND DISINFECTANTS

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ABSTRACT

Background: **Resistance of bacteria to antibiotics and disinfectants has been reported widely in the world. *Listeria monocytogenes* is no exception, although normally it tends to be variably sensitive to many antibiotics and disinfectants.**

Objectives: **To assess the susceptibility of *Listeria* isolates recovered from indigenous chickens to commonly used antimicrobials.**

Design: **Nine *Listeria* isolates recovered from village chickens were tested for sensitivity to commonly used antibiotics and disinfectants and compared with *Listeria monocytogenes* type strains (L028 and DGH), *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* ATCC 25922.**

Subjects: **Nine *Listeria* isolates.**

Interventions: **None.**

Main outcome measures: **Susceptibility to eight antibiotics and seven disinfectants.**

Results: **The nine *Listeria* isolates were sensitive to gentamycin (100%), kanamycin (88.9%), tetracycline (77.8%), cotrimoxazole (66.7%), chloramphenicol (66.7%) and resistant to ampicillin, augmentin and cefuroxime. There was no difference between the antibiotic sensitivity to the various *Listeria* isolates and *Listeria monocytogenes* type strains (P>0.05). The isolates were sensitive to disinfectants; A (100%), B (88.9 %), D (77.8%), E (77.8%) but resistant to, CF, and G. There was significant difference between the resistance of *Listeria* isolates to the various disinfectants at the varied dilutions and the resistance at the recommended user - dilution (P< 0.00293).**

Conclusion: **This study has shown that some of the *Listeria* isolates were resistant to most common antimicrobial agents except gentamycin and disinfectant A. Hence the need to consider this resistance pattern for effective treatment and control of listeriosis.**

INTRODUCTION

The increasing prevalence of bacterial resistance to commonly prescribed antibiotics and disinfectants especially in developing countries poses a major concern in the management of infections(1-3). This has mainly resulted from extensive use and often misuse of antimicrobials and disinfectants in both human and veterinary medicine(4,5). This is especially important as some drugs (gentamycin and tetracycline) are shared between human and veterinary medicine.

Work done on resistant strains of *Listeria monocytogenes* with respect to the heritability of the mutations indicated that several, but not all, of the mutations were heritable and were most likely located on the chromosome(6). The gene(s) may also be transferred through conjugative reproduction.

This study was therefore done to assess the susceptibility of *Listeria* isolates to commonly used antibiotics and disinfectants for effective treatment and control of listeriosis in village chickens.

MATERIALS AND METHODS

Bacterial strains used: Three types of cultures were used in this study. These were; *Listeria monocytogenes* type strains (L028 and DGH) from Denmark and *Staphytococcus aureus* NCTC 6571 and *Escherichia coli* ATCC 25922 from Kenya Medical Research Institute (KEMRI). *Listeria monocytogenes* type cultures were used for antibiotics and disinfectant sensitivity tests. The *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* ATCC 25922 were used as controls for antibiotic sensitivity tests. Nine *Listeria* isolates from chicken were also used.

Antibiotics and disinfectants used: The antibiotics that were used in the test included: ampicillin (25 µg); tetracycline (25µg); cotrimoxazole (25µg); augmentin (30 µg); kanamycin (30µg); gentamicin (10µg); cefuroxime (30µg) and chloramphenicol (30µg. Ampicillin, tetracycline and cotrimoxazole were in higher concentrations than the recommended minimum inhibitory concentration by NCCLS.

Seven commonly used disinfectants, referred to here as A, B, C, D, E, F, and G were selected for this study. The active ingredient for disinfectant A was, Glutaraldehyde and

Coco - benzyl dimethyl ammonium chloride; B was, Didecyldimethyl ammonium bromide 50% w\v; C was, Sodium hypochlorite; D was pine disinfectant and antiseptic; E was, chloroxylenol; F was, phenol and that for disinfectant G was, cresol and soap solution. Different dilutions for each disinfectant were used, namely: the user dilution, two - fold dilutions above and two fold dilutions below the user dilution (Table 1).

Antibiotic sensitivity testing of Listeria isolates: The test was done using modified controlled disk diffusion technique(7) on Mueller-Hinton agar (Oxoid limited, Basingstoke, Hampshire, England). The two *Listeria monocytogenes* isolates and the seven other *Listeria* species isolated were subcultured from egg yolk agar slants onto sheep blood agar and incubated aerobically at 37°C for 48 hours. Single colonies from respective isolates were then inoculated into brain - heart infusion broth (Oxoid, Basingstoke, and Hampshire, England) and incubated at 37°C aerobically for 18 hours.

To prepare the bacterial lawn on petri dishes, 150 microlitres of the bacterial suspension containing 10^7 Colony forming units (cfu) / ml were dropped onto each Mueller - Hinton agar plate and then spread out using a sterile glass rod. Antibiotic discs were then placed on the inoculated plates using a pair of sterile forceps, which were also used to gently press the discs onto the surface. The plates were then incubated at 37°C overnight and the diameters of each zone of inhibition, including the diameter of the disc, measured in millimetres. In the interpretation of the results, only those that showed full susceptibility as given by national clinical control of laboratory standards (NCCLS) tables (1997) were considered; hence the intermediate diameters were ignored.

Disinfectant sensitivity testing of Listeria isolates: A diffusion technique using wells punched into Mueller - Hinton agar was used, following the method of(8). The bacteria were seeded in the same way as antibiotic sensitivity testing. After seeding, wells were punched using a sterile 6 mm diameter well - cutter. Five different dilutions of 7 commonly used disinfectants were dispensed, using a micropipette, into different wells, using one petri - dish per disinfectant. Each well was loaded with 50µl of the relevant disinfectant dilution. The plates were then incubated upright at 37°c overnight and the diameters of each inhibition zone measured and recorded in millimetres. The results were interpreted as follows: inhibition zones of upto 2 millimetres from the edge of the well to the inhibition zone front (10mm diameter) were taken as resistant, and beyond 2 millimetres (10mm. diameter) were taken as sensitive.

RESULTS

Antibiotic sensitivity testing of Listeria monocytogenes and other Listeria spp: Figure 1 gives the sensitivity patterns for the nine Listeria isolates tested. All the isolates were resistant to ampicilin, augmentin and cefuroxime. They were 100% susceptible to gentamicin. The difference between percentage resistances of the *Listeria* isolates and the Listeria *monocytogenes* type strains was found to be insignificant.

Figure 2 gives the antibiotic multiple resistance patterns for the isolates tested. All the nine *Listeria* isolates showed multiple resistance to at least three antibiotics while a few were resistant to more than five antibiotics.

Table 1

Dilutions of the seven disinfectants

Disinfectant type	Dilutions (%)				
	1	2	3*	4	5
A	1	0.5	0.5	0.25	0.0625
B	0.2	0.1	0.05	0.025	0.0125
C	10	5	2.5	1.25	0.625
D	16	8	4	2	1
E	24	12	6	3	1.5
F	5.3	2.6	1.3	0.67	0.33
G	20	10	5	2.5	1.25

*= Recommended user dilution

Figure 1

Susceptibility of Listeria species to eight common antibiotics

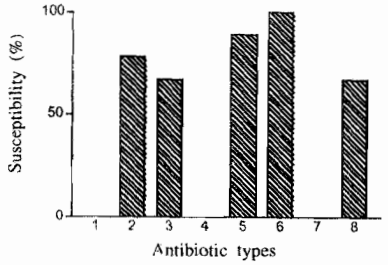


Figure 2

Frequency of multiple resistance to tested antibiotics among Listeria isolates

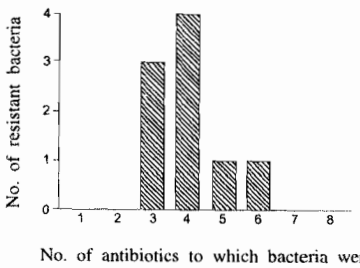
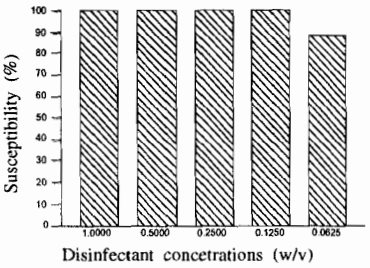


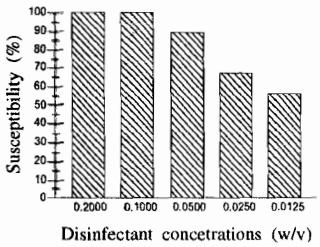
Figure 3

Disinfectants profile for Listeria Isolates

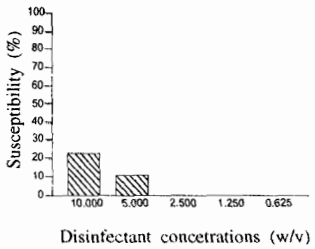
Disinfectant A



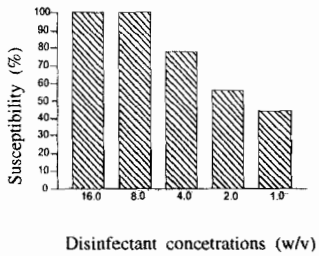
Disinfectant B



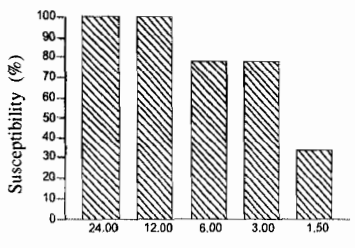
Disinfectant C



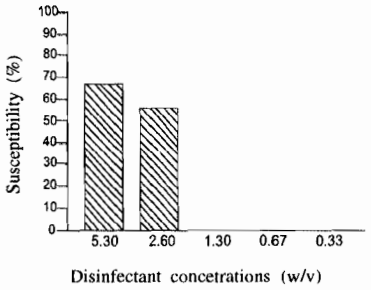
Disinfectant D



Disinfectant E



Disinfectant F



Disinfectant sensitivity testing on Listeria spp: Figure 3 gives the sensitivity patterns for the nine isolates tested, using the seven disinfectants. All the *Listeria* isolates tested were sensitive to disinfectant A at the recommended user dilution and also at lower dilutions. Eighty nine percent of the isolates tested were susceptible to disinfectant B at the recommended user dilution, 66.7% were sensitive at a higher dilution while all the isolates were 100% sensitive to B at a lower dilution than the recommended user - dilution. All the isolates were sensitive to D and E at lower dilutions than the recommended user - dilution, while 77.8% were susceptible to both disinfectants at the recommended user- dilution. All the isolates showed resistance to F at the recommended user - dilution, with more than 50% showing susceptibility at lower dilutions than the recommended user - dilution. All the isolates were resistant to disinfectant C at the recommended - user dilution, with only 22.2% to 11.1% being sensitive at dilutions lower than the recommended user- dilution. All the isolates were resistant to G at all dilutions used in this study. The relative sensitivities of the *Listeria* isolates to the disinfectants at various dilutions were found to be significant when compared to the recommended user- dilution ($P< 0.01$).

DISCUSSION

Antibiotics are used extensively worldwide, to treat bacterial diseases, including listeriosis(9-11). In this study, *Listeria monocytogenes* and other *Listeria* species isolated were found to be sensitive to gentamicin, while a few showed variable resistance to cotrimoxazole, tetracycline, kanamycin and chloramphenicol. All the isolates showed resistance to ampicillin, augmentin and cefuroxime. The *Listeria monocytogenes* type strains (L028 and DGH) strains showed a similar antibiotic sensitivity patterns to that of *Listeria* field isolates.

Unlike Poyart - Salmeron *et al*(12) who reported that *Listeria monocytogenes* was sensitive to penicillin, amoxycillin, gentamicin, chloramphenicol, trimethoprim, cotrimoxazole, erythromycin, vancomycin, rifampicin, imipenem and tetracyclines but resistant to most cephalosporins. The resistance could be through plasmid(13). Poyart Salmeron(12), have documented

this type of transmission in tetracycline, erythromycin, chloramphenicol and streptomycin. This drug resistance, single or multiple, has also been reported in Gram-positive bacteria by a number of workers(14,15). The fact that, the type strains and most of the Listeria isolates in this study showed multiple resistances, posses a problem in treating listeriosis in humans and domestic animals.

To clean and disinfect the chicken coops, houses, farmer's house and environs where chickens are kept, various disinfectants can be used(16,17). *Listeria* isolates were tested with seven common disinfectants. Of these, A was found to be the most effective disinfectant, followed by B, D and E while C and F were effective at concentrations higher than the recommended user dilution A and B were the only ones that were 85 - 100% effective at the recommended user dilution while other disinfectants required higher concentrations to achieve significant sensitivity. Disinfectant G had no effect at all the dilutions studied.

Listeria monocytogenes type cultures (L028 and DGH) gave reactions similar to the local isolates recovered in this study, when tested with disinfectants, C, E, F and G, but showed greater sensitivity to disinfectants A, B and D using both the recommended user- dilution and lower ones. Bebora and Karaba(8,18) tested *Salmonella gallinarum* against B, F, G, Pynol - 5, Biodan and Municipal fluid, found F and G not effective at the recommended user dilution. These observations emphasise the importance of ascertaining the efficacy of a disinfectant on a respective organism for one to effectively eliminate it from the disinfected area.

This study has shown that *Listeria monocytogenes* and other *Listeria* species have some degree of resistance to some of the commonly used antibiotics and disinfectants and will require higher concentration than the recommended user - dilution for effectiveness. Disinfectants A and B were found to be the most effective at user - dilution. Other disinfectants will need to be used at higher concentrations than the user dilution.

In conclusion, there is widespread *Listeria* species resistance against antimicrobials, which limits the effective control and management of poultry infections. Further investigations are required to investigate the cause of this resistance, which may be in form of plasmids that may carry genes for antimicrobial resistance and their transmission between these bacteria.

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