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SUSCEPTIBILITY OF ESCHERICHIA COLI; SALMONELLA PULLORUM AND SALMONELLA GALLINARUM TO CHLORAMPHENICOL IN VITRO

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SUMMARY

Escherichia coli, Salmonella pullorum and Salmonella gallinarum were tested for susceptibility to Chloramphenicol by tube broth dilution technique and disk-diffusion method. MICS and Zone diameter were correlated with production of chloramphenicol acetyltransferase (CAT). Also Evaluation of a 30-minutes commercial disk procedure demonstrated that, it is as accuratc as 1-hour tube method for detection of resistance among these strains.

INTRODUCTION

Avian salmonellosis and colibacillosis are problems of economic concern to all phases of poultry industry from production to marketing. The emergence of chloramphenicol resistance among *Salmonella* spp. and *Escherichia coli* has been an important public health problem. The mechanism of resistance among most strains is due to the production of chloramphenicol acetyltransferase enzyme which is mediated by R plasmids among these strains (Gaffney and Foster, 1978).

Since its discovery in 1947, chloramphenicol has been widely used as therapy for *Salmonella* and *Escherichia coli* infections and is the treatment of choice for Avian salmonellosis and colibacillosis, because of its easy administration and its clinical efficacy. Because of rare potential adverse reactions (Smith and Weber, 1983), its use has been limited to those life - threating infections. However, in Egypt Chloramphenicol remains the antimicrabial agent of choice for serious *Salmonella* and *Escherichia coli* infections as well as a variety of other syndromes.

The intent of the present investigation was to evaluate the adequacy of two rapid screening methods for the detection of chloramphenicol resistance among *Salmonella* and *Escherichia coli* strains and to correlate it to the production of chloramphenicol acetyltransferase (CAT).

MATERIALS AND METHODS

Seventy strains were studied: *Escherichia coli* (n=32) were isolated from cases of colibacillosis, *Salmonella pullorum* (n=18) from cases of pullorum disease and *Salmondella gallinarum* n=20) from cases of fowl typhoid. The isolates were identified using the biochemical and serological tests according to Sonnenwirth (1980) Strains were inoculated onto Mac-Conkey agar media, and were incubated at 35°C in an ambient atmosphere for 24 hours. Thereafter, a single colony was transferred to a second blood agar plate and incubated at 35°C for 18 hours, growth from this plate was used to prepare inocula for all subsequent tests.

MICs were determined by use broth dilution technique as recommended by Matsen (1980). In brief, two fold dilutions of Chloramphenicol (Parke-Davis, Morris Plains, N. J.) in cation-supplemented Mueller-Hinton broth were tested with concentrations ranging from 0.125 to 256.0µg/ml. The MIC was defined as the lowest concentration of antibiotic that did not allow macroscopic evidence of growth after 18 hours. The final inoculum in each tube was approximately 2 x 10^5 CFU per ml. The size of

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the inoculum was confirmed periodically throughout the study by colony counts. All determinations were performed in duplicate.

CAT activity was investigated by a 1-hour visual tube CAT (t-CAT) assay performed as described by Azemun et al., (1981), using the reagents sodium dodecyl sulfate, EDTA, trizma Hcl, trizma base, acetyl coenzyme A, Sodium chloride and 5.5-di-thiobis (2- nitrobenzoic acid). In addition, a 30 minutes disk CAT (d-CAT) assay was evaluated by using a commercially CAT reagent-kit (Remel, Lenexa, Kans.) with some modifications to the method of the manufacturer. In brief, the strains were grown overnight on solid media impregnated with 30 µg chloramphenicol disks (for induction of the enzyme). Cells were taken from around a disk with a loop to make cell suspension in 5 ml of physiologic saline. which is used for d-CAT assy.

The tubes were incubated at 35°C for 30 minutes, and reaction was evaluated by comparing the colour in control tube with that in the experimental tube. A colour range from pale yellow to deep yellow indicated CAT activity.

RESULTS

The susceptibility levels of *Escherichia coli*, *Salmonella pullorum* and *Salmonella gallinarum* to chloramphenicol are shown in table (1). On the basis of zone size, none of the chloramphenicol - susceptible strains of *Escherichia coli* were producers of CAT, while, 12 CAT- positive strains were resistant by zone size and MIC with modes of 15 mm and 16 µg/ml. respectively.

For *Salmonella pullorum* 13 out of 18 strains were both CAT negative and susceptible to chloramphenicol by zone size, mode 30 mm and MIC, mode 2.0 µg/ml. while all the resistant straine were CAT-positive.

Of 20 *Salmonella gallinarum* strains tested for susceptibility to chloramphenicol 16 were susceptible by zone size, mode 28mm and MIC, mode 0.5 µg/ml, while 4 strains were CAT-positive and resistant to chloramphenicol by both zone size and MIC. modes 10 mm and 8 µg/ml respectively.

The d-CAT and the t-CAT detection method yielded identical results for all the isolated strains with some distinct degree of colour change produced by resistant strains.

Table (1): Susceptibility of *Escherichia coli*, *Salmonella pullorum* and *Salmonella gallinarum* to chloramphenicol.

Organism and chloramphenicol susceptibility	No. of strains Tested	CAT activity		Zone size (mm)		MIC (µg/ml)	
		t-CAT	d-CAT	range	mode	range	mode
Escherichia coli							
Susceptible	20	-	-	21-30	23	0.5 - 4.0	2.0
Resistant	12	+	+	9-18	15	16 - 64	16
Salmonella pullorum							
Susceptible	13	-	-	26-32	30	1.0 - 4.0	2.0
Resistant	5	+	+	8-17	9	8.0 - 32	16
Salmonella gallinarum							
Susceptible	16	-	-	24-39	28	0.5 - 2.0	0.5
Resistant	4	+	+	9-15	10	8.0 - 16	8.0

DISCUSSION

In 1980 Masten established MIC and zone diameter breakpoints for chloramphenicol : ≥ 25 µg/ml and ≤ 12 mm respectively for resistant and ≤ 12.5 µg/ml and ≥ 18 mm respectively for susceptible strains. These breakpoints proved to be problematic when *Escherichia coli. Salmonella pullorum* and *Salmonella gallinarum* strains were tested for chloramphenicol resistence by relating in vitro susceptiblity results to CAT production.

In this paper the susceptibility of *Escherichia coli, Salmonella pullorum* and *Salmonella gallinarum* to chloramphenicol was investigated, also evaluation of the adequacy of two rapid screening methods for the detection of chloramphenicol resistance in *Escherichia coli* and *Salminella* species was performed.

Also a new breakpoints that might better assess the relationship between CAT production and chloramphenicol resistance in these strains was recommended.

I suggest that the MIC and zone diameter breakpoints for *Salmonella pullorum* and *Salmonella gallinarum* can be the same (≥ 8 µg/ml and ≤ 17 mm, respectively for resistant and ≤ 4.0 µg/ml and ≥ 21 mm respectively for susceptible strains.

However, the breakpoints for *Escherichia coli* must be different (≥ 16 µg/ml and ≤ 18 mm for resistant and ≤ 4.0 µg/ml and ≥ 21 mm. for susceptible).

By suing two rapid methods to assay CAT production by *Escherichia coli, salmonella pullorum* and *Salmonella gallinarum*, 100% correlation will be obtained.

Based on these data; it appears that the d-CAT method is useful for sensitive and rapid detection of chloramphenicol resistance in *Salmonella* and *Escherichia coli* species. This method could be used in areas of the world where chloramphenicol remains the treatment of choice for serious infections. Although the manufacturer of the disk used in this study for detection of CAT activity recommends it for *Haemophilus influenzae*, but it can also be used to detect CAT activity in *Salmonella* species and *Escherichia coli* even though the intensity of the colour in the positive test is not as great as with the tube test.

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