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MICROBIAL AGENTS INCREMINATED IN REDUCED HATCHABILITY OF DUCK EMBRYOS II. ANTIMICROBIAL IN-VITRO SENSITIVITY TESTING OF *Salmonella emek* AND *E. coli* ISOLATES USING MINIMUM INHIBITORY CONCENTRATION (MIC)

(With 2 Tables)

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العوامل البكتيرية المتهمة فى إنخفاض معدل الفقس فى أجنة بيض البط ٢ - إختبار حساسية المضادات الميكروبية ضد معزولات ميكروب السالمونيلا ايميك والميكروب القولونى باستخدام أقل جرعة مثبطة فى الآجار

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تم اختبار حساسية ١٣ معزولة من ميكروب السالمونيلا ايميك و ١١ معزولة من الميكروب القولونى ضد عشرة أنواع من المضادات الميكروبية باستخدام اختبار أقل جرعة مثبطة. بخصوص السالمونيلا ايميك أظهر ٩٢,٣% من العترات مقاومة السلفاديميدين وكانت أقل جرعة مثبطة أقل من أو تساوى ٥٠ ميكروجرام لكل مللى بينما كانت كل العترات خاضعة تماما لتأثير الفلوميكوين والانرو فلوكساسين واموكساسيللين واوكسى تتراسيكلين وكلورتتراسيكلين واريثروميسين ونيوميسين وحمض الناليديكس والكولستين بأقل جرعة مثبطة ٠,٢ ، ٠,٢ ، ٠,٢ ، ٠,٤ ، ٠,٤، ٠,٧٨ ، ٠,٧٨ ، ١,٥٦ ، ١,٥٦ ميكروجرام لكل مللى على التوالى. لوحظت أربعة أشكال من المقاومة بالنظر الى معزولات الميكروب القولونى عند إجراء اختبار الحساسية. وهذه الاشكال كالتالى: الكولستين بمعدل ١٨,٢% بأقل جرعة مثبطة ٦,٢٥ ميكروجرام لكل مللى، حمض الناليديكس بمعدل ٥٤,٥% بأقل جرعة مثبطة تراوحت من ٣,١٣ الى ٦,٢٥ ميكروجرام لكل مللى ومقاومة بنسبة ١٠٠% ضد الاموكساسيللين والسلفاديميدين بأقل جرعة مثبطة ٦,٢٥ ، ٥٠ ميكروجرام لكل مللى على

التوالى. وخضعت كل معزولات الميكروب القولونى لتأثير الانروفلوكساسين (٠.٢ ميكروجرام مللى) والكلوتتراسيكلين والاوكسى تتراسيكلين (٠,٤ ميكروجرام / مللى) اريثروميسين (٠,٧٨ ميكروجرام / مللى)، نيوميسين ٠,٤ ~ ٠,٧٨ ميكروجرام / مللى) والكلولستين (١,٥٦ ميكروجرام / مللى).

SUMMARY

Thirteen isolates of *Salmonella emek*, and Eleven isolates of *E. coli*, were tested for their susceptibility to ten antimicrobial agents using MIC technique. Concerning *Salmonella emek*, 92.3% of isolates were resistant to the action of sulfademidine with MIC value of ≥ 50 μg/ml. All isolates were completely susceptible to the action of flumequine, enrofloxacine, amoxacilline, oxytetracycline, chlortetracycline, erythromycin, neomycin, nalidixic acid and colistin sulphate with MIC values of 0.2, 0.2, 0.2, 0.4, 0.4, 0.78, 0.78, 1.56 and 1.56 μg/ml respectively. Four resistance patterns were noticed regarding *E. coli* isolates sensitivity testing. Colistin sulphate (18.2%) with MIC value of 6.25 μg/ml, nalidixic acid (54.5%) with MIC value ranged from 3.13 ~ 6.25 μg/ml, followed by 100% resistance of amoxacillin and sulfademidine with MIC values of 6.25 and 50 μg/ml. All isolates were completely susceptible to the action of enrofloxacine (MIC ≥ 0.2 μg/ml), chlortetracycline and oxytetracycline (MIC ≥ 0.4 μg/ml), erythromycin (MIC ≥ 0.78 μg/ml), neomycin (MIC range of 0.4 ~ 0.78 μg/ml) and colistin sulphate (MIC ≥ 1.56 μg/ml).

Key Words: *Antimicrobial, Susceptibility, MIC, Enrofloxacine, Resistance, Salmonella emek, E.coli*

INTRODUCTION

Antibiotics are used therapeutically and for prophylaxis in intensive poultry farming. However, strains of bacteria resistant to antibiotics emerge, even under controlled use of antibiotics (Cloud et al., 1985). Reduction of decreased hatchability of fertile eggs may be improved by egg treatment or breeding duck treatment with antibiotics (El-Gharib, 1991). The effective antimicrobial agents required for egg or breeder duck treatment should be selected and tested carefully to ensure better results and to avoid the use of ineffective antimicrobials. This work was carried out to test the different antimicrobial agents sensitivity against *Salmonella emek* and *E. coli* isolated from infertile duck eggs and dead in-shell duck embryos.

MATERIALS and METHODS

Bacterial strains: Eleven isolates were biochemically typed as *E. coli* and thirteen isolates were typed biochemically and serologically as *Salmonella enteritidis ser emek*. These isolates were isolated from unhatched duck embryos and tested for minimum inhibitory concentration (MIC) of ten antimicrobial agents.

Antimicrobials dilution: Ten antimicrobial agents of 100% potency and kindly provided by AMOUN, Egypt. Those antimicrobials are quinolons (nalidixic acid, flumequine, enrofloxacine), neomycin, colistin sulphate, chlortetracycline, oxytetracycline, erythromycin, amoxacilline and sulfademidine.

Serial two fold dilutions of the aforementioned antimicrobial agents were carried out in sterile distilled water in the range of 0.1 to 50 μg/ml according to individual MIC breakpoint of each antimicrobial agent. The stock solutions of antimicrobials were made in distilled water except for oxytetracycline and chlortetracycline, were done in ethanol.

Bacterial culture preparation: The bacterial isolates were outplated on dextrose starch agar (DSA, Difco) plates and incubated at 37°C overnight. A single colony was picked and inoculated into 5 ml of tryptose broth (TB, Difco) and incubated at 37°C in shaking water bath for 6 hours.

MIC test procedure: The agar dilution method was used for determination of MIC values of each antimicrobial agent. Each antimicrobial dilution was mixed with molten DSA at 40°C and poured onto sterile plates. A 10^{-2} dilution of 6 hours TB culture was inoculated by micropipette into DSA plates containing serial two fold dilutions of different antimicrobials. The agar plates were left to dry and incubated at 37°C for 25 hours. The MIC was defined as the lowest concentration of antimicrobial agent that prevented bacterial growth, (Mitsuhashi et al, 1981).

RESULTS

Thirteen isolates of *Salmonella emek* and Eleven isolates of *E. coli*, their antimicrobial susceptibility for ten antimicrobial agents using MIC techniques revealed the following results:
In case of *Salmonella emek*: Twelve out of 13 isolates resisted the action of sulfademidine (92.3%) with MIC value of 50 μg/ml, while only one isolate was susceptible with MIC value of 3.13 μg/ml (7.6%). A highest susceptibility was for enrofloxacine (MIC = 0.2 μg/ml), flumequine (MIC = 0.2 μg/ml), amoxacilline (MIC = 0.2 μg/ml), chlortetracycline and oxytetracycline (MIC = 0.4 μg/ml), erythromycin, neomycin (0.78 μg/ml), colistin sulphate and nalidixic acid (MIC = 1.56 μg/ml). Results are shown in Table 1. In case of *E. coli*: There are four resistance patterns. These patterns are colistin sulphate resistance (18.2%) with MIC of 6.25 μg/ml, nalidixic acid resistance (54.5%) with MIC $\geq$ 6.25 μg/ml, amoxacilline resistance (100%) with MIC $\geq$ 6.25 μg/ml and sulfademidine resistance (100%) with MIC $\geq$ 50 μg/ml. The highest susceptibility were for enrofloxacine (MIC = 0.2 μg/ml), chlortetracycline and oxytetracycline (MIC = 0.4 μg/ml), followed by erythromycin, neomycin, flumequine and colistin sulphate with MIC values of 0.78, 0.78 ~ 1.56, 0.4 ~ 0.78 and 1.56 μg/ml respectively. Results are shown in Table 2.

DISCUSSION

In-vitro susceptibility testing was done on *Salmonella emek* and *E. coli* isolates to determine the most effective antimicrobial agents. Regarding to antimicrobial susceptibility of *Salmonella emek*, our results revealed that only drug resistance was for sulfademidine (92.3%; MIC $\geq$ 50 μg/ml), while all isolates were varied in their susceptibility to other antimicrobial agents. The most effective drugs were enrofloxacine and flumequine (MIC $\geq$ 0.2 μg/ml), followed by amoxacilline, chlortetracycline, oxytetracycline, erythromycin, neomycin, nalidixic acid and colistin sulphate with MIC range of 0.2 ~ 1.56 μg/ml. Sensitivity of *Salmonella* and *E. coli* species to ampicilline, erythromycin, tetracycline, enrofloxacine and sulfademidine was investigated by Watts et al. (1993) and revealed that enrofloxacine was the most active drug against these bacteria and supporting our findings. On opposite side Cicek and Kovarik (1994) found *Salmonella enteritidis* with multiple drug resistance to tetracycline, ampicilline and colistin. Tracing the available literatures back, only Matsushita et al. (1996) who described *Salmonella emek* as imported strain with high resistance rate (83.3%). The resistance patterns were to TC.SM, TC, CP.TC.SM.ST, TC.SM.KM. and CP. TC.SM.KM contrasting to our results in this respect. Different *Salmonella serovars* resistance to neomycin, ampicilline, sulfamethoxazole, and tetracyclines were reported by Poppe et al. (1995).

On studying the antimicrobial sensitivity of *E. coli* isolates from duck unhatched eggs, we found several antimicrobial resistance. Colistin resistance (18.2%) with MIC = 6.25 μg/ml, nalidixic acid resistance (54.5%) with MIC value of 6.25 μg/ml and 100% resistance for both amoxicilline (MIC $\geq$ 6.25 μg/ml) and sulfademidine (MIC $\geq$ 50 μg/ml) were noticed in the present work. The highest susceptibility for flouroquinolone (enrofloxacine) with MIC = 0.2 μg/ml, followed by tetracyclines, erythromycin, neomycin, flumequine and colistin sulphate.

In this work, variations in resistance pattern among isolates of the same serovars, may refered to obtaining hatching eggs from various sources.

Nalidixic acid is the first generation of quinolones. Griggs et al. (1994) recovered nalidixic acid resistant isolates of *Salmonella* from veterinary sources. These isolates also found to be less susceptible to flouroquinolones and MIC range was increased. The authors suggested that increasing incidence of quinolone-resistance is a matter for concern. The most important point of view was developing of resistant mutants of bacteria, Bazile et al. (1996) isolated quinolone-resistant *E. coli* strains from poultry with past history of flumequine treatment. They could induce in vitro selection of ciprofloxacin resistance, suggesting more resistant mutants as well as cross-resistance between older quinolones and flouoroquirolones, consequently great care should be given to the random use of such drugs. In the present study, the authors found a similar results regarding to nalidixic acid resistance.

On opposite view, Reddy et al. (1995) found all isolates of *E. coli* which are identified as primary or secondary pathogen were sensitive to nalidixic acid and some isolates were sensitive to defloxacine and flumequine respectively.

Ampecilline, amoxacilline and sulfonamides resistance by *E. coli* isolates were reported by Filali et al. (1988), Mishra (1991), Babila and Akcadag (1992), Mishra (1995) and Tsubokura et al. (1995) confirming our results. Raemdonck et al. (1992) mentioned that flouroquinolone is the most potent drug for treatment *E. coli* from poultry. These results come in agreement to our findings.

The rampant use of antibiotics in treatment and as feed additives, made the bacteria resistant to different antibiotics at different times and at different places (Moharona et al., 1993). The continuos drug application well increase the MIC range of the same drug category (Kobe et al., 1995).

In summary, sulfademidine resistant *Salmonella emek* as well as colistin, nalidixic acid, amoxacilline and sulfademidine resistant *E. coli* isolates were recovered from unhatched duck embryos. This is may be due to continuos use of drugs, leading to increase of MIC value and consequently, appearance of resistant bacteria to those drugs and related antimicrobials. Such resistant mutants are of very important value in explanation of drug treatment failure and may bring us to the end of magic era of antibiotics.

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Table 1. MIC of 13 *Salmonella emek* isolates against 10 antimicrobial agents.

Antimicrobial agent	Minimal Inhibitory Concentration (µg/ml)										MIC break-point of resistance	No. of resistant strains
	0.1	0.2	0.4	0.78	1.56	3.13	6.25	12.5	25	50		
Enrofloxacine		13									12.5	-
Nalidixic acid					13						6.25	-
Flumequine		13									6.25	-
Colistin					13						6.25	-
Erythromycin				13							6.25	-
Neomycin				13							12.5	-
Amoxacilline		10	3								6.25	-
Chlortetracycline			13								25	-
Oxytetracycline			13								25	-
Sulfademidine						1				12	50	12

Table 2. MIC of 11 *E.coli* isolates against 10 antimicrobial agents.

Antimicrobial	Minimal Inhibitory Concentration (µg/ml)										MIC break-point of resistance	No. of resistant strains
	0.1	0.2	0.4	0.78	1.56	3.13	6.25	12.5	25	50		
Enrofloxacine		11									12.5	-
Nalidixic acid						5	6				6.25	6
Flumequine			8	3							6.25	-
Colistin					9		2				6.25	2
Erythromycin				11							6.25	-
Neomycin				8	3						12.5	-
Amoxacilline							11				6.25	11
Chlortetracycline			11								25	-
Oxytetracycline			11								25	-
Sulfademidine										11	50	11