

ANTIMICROBIAL DRUGS SUSCEPTIBILITY OF SOME BACTERIAL PATHOGENS ISOLATED FROM NATURALLY INFECTED PSITTACINE BIRDS IN SHARKIA PROVINCE

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ABSTRACT

Thirty eight bacterial agents were isolated from different species of psittacine birds of variable ages. These birds included morbid and/ or dead birds. Birds were received from different localities of Sharkia province. These agents were *E. coli* (31.6%), *Shigella* spp. (21%), *Salmonella* spp. (15.8%), each of *Citrobacter* spp. and *Enterobacter* spp. (10.5%) and each of *Proteus* spp. and *Klebsiella* spp. (5.3%). Susceptibility of these isolates to antimicrobial agents including fluoroquinolones (enrofloxacin, ciprofloxacin, difloxacin and norfloxacin) and other commonly used antimicrobials (gentamicin, streptomycin, flumequinone, nalidixic acid, amoxicillin, trimethoprim, tetracycline, doxycycline, penicillin and cefixime) were tested in-vitro by using disc diffusion and broth dilution techniques. All isolates were sensitive to enrofloxacin, ciprofloxacin and difloxacin with MICs ranged from 0.19-0.39, 0.19-0.78 and 0.39-1.56 ug/ml respectively. The MBCs were equal to or one doubling dilution above MICs. Norfloxacin and gentamicin were the next most active compounds with MICs ranged from 0.39- 6.25 and 0.39-12.5 ug/ml, respectively. Whereas, nalidixic acid, doxycycline, cefixime, and tetracycline exhibited variable activity against isolated bacteria. Moreover, *Proteus*, *Citrobacter* and *Enterobacter* were highly resistant to all tested drugs except fluoroquinolone compounds and doxycycline. Most of isolated strains were resistant to amoxicillin, flumequinone, streptomycin, trimethoprim and penicillin. These results provide information on the gram - negative bacteria isolated from psittacine birds, their drug susceptibility and the MICs and MBCs. This knowledge may prove useful to the clinician when selecting the appropriate antimicrobial agents to treat bacterial infection in psittacine birds.

INTRODUCTION

Birds belonging to the order psittaciforms are becoming increasingly more popular as house held pets. One of the diagnostic tools to investigate the cause of disease is a microbiological examination of fecal or samples taken from body openings like cloaca, nose and beak (**Dorrestein et al., 1985**). At necropsy, culturing from the intestine and different organs as liver, heart and lungs (**Flammer and Drewes, 1988**) can collect valuable information. The bacterial infections of psittacine birds either primary or secondary result in major economic losses in captive birds (**Bangert et al., 1988**). Gram - negative bacteria do not belong to the normal intestinal flora (**Glunder and Martinsen., 1981**). Any gram - negative bacterium cultured from the droppings can be considered a pathogen (**Flammer and Drewes.,1988 and Gerlach, 1994**). It is well established that in-vitro antibacterial susceptibility testing of bacterial pathogens can provide valuable guidance to the veterinarians in choice of appropriate antimicrobial agents. Some studies referred to strong correlation between in-vitro and in-vivo efficacy of antimicrobials (**Raemdonck et al., 1992**). Studies have reported susceptibility data for organisms isolated from chickens, turkeys and ducks (**Amara et al 1995, Watts, et al., 1993, Salmon and Watts, 2000**). However, very little data are available reporting the in-vitro activity of various antimicrobial agents against psittacine birds pathogens.

The objective of this study was to determine the in vitro activity of various antimicrobial agents by using disc diffusion and broth dilution techniques against gram-negative bacteria isolated from psittacine birds.

MATERIAL & METHODS

Birds :

Fifty psittacine birds were collected from different localities at Sharkia province with different ages. These birds were subjected to clinical and postmortem examination.

Collection of samples and microbiological examination :

Cloacal and tracheal swabs from morbid birds were taken as well as specimens from internal organs were collected at necropsy for bacteriological examination. Collected swabs and organs were kept in refrigeration before seeding in suitable media. These media included Nutrient broth and agar, MacConkey broth and agar, Selenite -F-broth, Eosin Methylene Blue agar, and S.S agar. (Difco). Inoculated culture media were incubated at 37°C for 24 - 48 hr. under aerobic conditions.

Subcultures were made from Selenite - F- broth to specific media S.S agar. Bacterial isolates were identified by growth characteristics, colonial morphology, gram s stain, and standard biochemical tests (Avery., 1982).

Drug susceptibility tests:

A- Disc diffusion test: A total of 38 isolates were used for disc sensitivity testing according to National Committee for Clinical Laboratory Standards (NCCLS) **procedures (1997)**. The antimicrobial discs used were enrofloxacin (Enr) 5 ug, difloxacin (Dif) 5 ug, ciprofloxacin (Cip) 10 ug, norfloxacin (Nor) 10 ug, Ceftriaxone (Cef) 10 ug, gentamicin (Gn) 10 ug, amoxicillin (Ami) 10 ug, penicillin (P) 10 ug, streptomycin (S) 10 ug, doxycycline (D) 30 ug, tetracycline (Tc) 30 ug, nalidixic acid (Na) 30 ug, Flumequine (Ar) 10 ug and trimethoprim (Trim) 25 ug (Oxoid, Unipath Ltd, Basingstoke, U.K.). The inhibition zones were measured after 18 to 24hr of growth and the recommendation given by the sensitivity discs manufacturer manual were used for classifying these isolates as sensitive or resistant.

B) Determination of minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs).

MICs and MBCs of antimicrobial agents for isolated strains were determined by using broth dilution method according to **NCCLS, (1997)**. Ten antimicrobials were kindly supplied by Anoun and Adwia Comp., Egypt were used in this study. These antimicrobials included enrofloxacin (Enr), ciprofloxacin (Cip) norfloxacin (Nor), difloxacin (Dif), streptomycin (S), gentamicin (Gm), nalidixic acid (Na), flumequine (Ar), amoxicillin (Ami) and tetracycline (Te). Selection of these antimicrobials were based on marked sensitivity of the bacterial agents and being in common use in the field.

The stock solutions of antimicrobials were made in sterile distilled water except for nalidixic acid, flumequine, enrofloxacin and ciprofloxacin which were dissolved in NaOH. A serial two fold dilution of each antimicrobial agent was done in the range of 0.05 - 100 ug/ ml in Mueller Hinton Broth (MHB). 10 ul of (MHB) containing 1×10^5 Colony Forming Unit (C.F.U) of each isolate was inoculated into each test and control tubes in the given series. The tubes were incubated at 37°C for 18 - 24 hr. The MIC was defined as the lowest concentration of the antimicrobial agent which completely inhibited the bacterial growth. Determination of Minimum Bactericidal Concentration (MBC) was done by subculturing (10 ul) of broth from each MIC tubes that had no visible growth on Mueller Hinton Agar (MHA) and incubated for 24 - 48 hr at 37°C. MBC was defined as the lowest concentration of a drug that showed no growth on the inoculated plate.

RESULTS AND DISCUSSION

Bacterial infections are frequent in psittacine birds. It is a common practice to culture from mouth and cloaca swabs as well as fecal samples routinely. Veterinarians use the results of these cultures to guide therapeutic decisions. The birds were suffered from depression, ruffled, plumage, nasal discharge, watery eyes and abnormal respiratory sounds, on the other hand some of them showed diarrhea, wet feathers at the vent region, and dehydration. Postmortum examination revealed that watery or mucoid nasal discharge, congestion of larynx and trachea, pneumonia and airsacculitis, the lesions in case of birds which suffered from diarrhea included congestion of the liver with subcapsular haemorrhage, necrosis in some cases, enteritis, and the content of the intestine were greenish dark material and in some cases were haemorrhagic contents. Kidney enlarged, and some birds suffering from splenomegaly.

The incidences of gram - negative bacteria are summarized in table (1). Seven genera of gram negative bacteria were isolated *E. coli* 12 (31.6%) was the most common, *Shigella* spp. 8 (21%), *Salmonella* spp. 6 (15.8%) *Citrobacter* spp 4 (10.5%) *Enterobacter* sp 4 (10.5%), *Proteus* spp. 2 (5.3%) and *Klebsiella* spp 2 (5.3%). No other species of gram - negative bacteria were recovered. **Graham and Graham (1978) and Dorrestein et al. (1985)** stated that all gram-negative bacteria are abnormal inhabitants of the psittacine gut and should be considered pathogens. In contrast, **Flammer and Drewes (1988)** noted species - related differences in the prevalence of gram - negative bacteria. They isolated *E. coli* from healthy psittacine birds in 60% (101/168) of the genus *Coccyzinae*. And 18% (61/338) from other noncocalinae species.

Caroline et al, (1999) suggested that shigatoxin - producing *E. coli* were uncommon in psittacine birds but enteropathogenic *E. coli* (EPEC) should be considered as potential pathogens in psittaciform birds, which may be a source of human EPEC infection. *E. coli* infection is probably the most common cause of death in psittacine birds in which it produces enteritis, pneumonia and septicemia (**Gerlach, 1994 and Steiner and Davis, 1981**).

Salmonellosis can be a serious disease of psittacine birds (**Coleman, 1993, Orosz et al, 1992 and Panigrahy and Gilmore, 1983**) the absence of fully functioning cecum in psittacines might explain why these birds appear to be more susceptible to salmonellae infections than other birds (**Gerlach, 1994**).

Enterobacter has been known to occasionally cause disease in psittaciforms (**Fiennes, 1982 and Gerlach, 1994**). **Dorrestein et al., (1985)** isolated *Klebsiella* in association with other bacterial agents from diseased birds but in mixed culture, dead birds showed on necropsy catarrhal to fibrinous pneumonia with airsacculitis. In contrast **Flammer & Drewes (1988) and Bangert et al. (1988)** isolated *Klebsiella* spp and *Enterobacter* spp from healthy birds. While *Citrobacter*

spp was isolated in a pure culture from different organs with gastroenteritis. They also isolated *Enterobacter* spp from organs including heart, kidney, lungs and gut. *Proteus* spp. and *Citrobacter* spp couldn't be isolated as a primary cause of the disease but were found in combination with other bacteria especially *E. coli*.

The in vitro activities of fluoroquinolones and other commonly used antimicrobial agents against isolated microorganisms are shown in Tables (2 and 3)

The selection of antimicrobial agents depends on knowledge of the susceptibility of the suspected pathogens to antibiotics, as well as effectiveness and length of drug withdrawal time (Prescott and Yelding, 1990).

Antimicrobial susceptibility testing is generally accepted for use as a guide to choice of antibiotic for therapy of psittacine birds (Flammer and Drewes, 1988 and Scullion, 1989). Data derived from disc diffusion tests are of value because they distinguish between sensitive and resistant strains in a given bacterial population and prediction of optimal therapy on the basis of pharmacokinetic and MICs data (Flammer, 1995).

Pharmacodynamic is defined as the correlation between concentration of the antibacterial agent and the effect of that agent on the bacterial pathogens. Initially, the pharmacodynamic properties of clinical concern were MIC and MBC. If the MBC and MIC were approximately the same or two-four dilution greater, the drug was considered to be bactericidal, on the other hand, if the MBC was several dilution greater than MIC the drug was considered to be bacteriostatic (Walker and Thomsberry, 1998 and Craig and Dalhoff, 1998).

In the current study, most of isolated strains were highly sensitive to enrofloxacin, ciprofloxacin and difloxacin with MICs ranged from 0.19 - 0.39, 0.19 - 0.78 and 0.39 - 1.56 µg/ml respectively, meanwhile, MICs of norfloxacin was slightly higher 0.39 - 6.5 µg/ml. The MBCs of these drugs for the most isolated bacteria were equal to or one doubling dilution above MICs revealing that these drugs possess bactericidal effect against the tested bacteria. These results were broadly similar to the previously published surveys in chicken. Saleem et al (1999) reported that all isolates of *E. coli* were sensitive to enrofloxacin and ciprofloxacin whereas, 98% of the isolates displayed sensitivity to norfloxacin, also difloxacin was highly effective against *E. coli* with MIC and MBC 0.312 - 1.25 µg/ml respectively (El-Azzawy and Khodary, 2003). In pigeons *Salmonella* spp. were completely sensitive to enrofloxacin with MIC values ranged from 0.78 - 1.56 µg/ml (Ibrahim et al., (2001). Balley et al., (1998a) found that in bustards all microorganisms were sensitive to enrofloxacin with MIC 50 and MIC90 (0.5 : 1.5 µg/ml respectively).

Moreover, the clinical efficacy of the antimicrobial agents is dependent on the serum concentration of the drug in relation to the MIC of the pathogen, the higher concentration of the drug

above the MIC of the pathogen, the greater bactericidal effect and the less the likelihood of selecting resistant organisms (**Walker and Thomsberry 1998**). Pharmacokinetic investigation in bustards demonstrated plasma enrofloxacin concentration exceeded 0.5 ug/ ml after administration of 10 - 15 mg/kg B.W for 12-24 hr suggesting the usefulness of this agent in the therapy of bacterial infection (**Bailey et al, 1998 b**).

In the present study the MICs and MBCs for fluoroquinolones specially for norfloxacin were slightly higher than previously reported in ducks (**Watts et al., 1993**), chickens (**Khodary and Ablam, 1997**), and turkey (**Salmon and Watts, 2000**) which probably, may reflect the previous use of these drugs in veterinary and human medicine and development of strains resistant to fluoroquinolones.

Resistance in gram-negative bacteria to fluoroquinolones is achieved by alteration in both the A and B fractions of DNA - gyrase (**Cullman, 1990**). The use of one fluoroquinolone may inactivate the other (**Chu and Fernandes, 1989, Cullman, 1990**). In fact, cross resistance between fluoroquinolones has been described (**Malorny et al., 1999**). With appropriate use of newer fluoroquinolones (enrofloxacin, ciprofloxacin and difloxacin), it may be possible that bacterial resistance should develop more slowly than it would with norfloxacin, this is due to a combination of factors, pharmacokinetic variables, antibacterial potency and the fact that the newer fluoroquinolones (with both, an ethyl - piperazynil group in position 7 and cyclopropyl group in position 1 of the quinolonic ring) and have 4 sites of action (2 subunits A and 2 B) in the topoisomerase II enzyme (**Cullman, 1990**). Whereas early developed fluoroquinolones such as norfloxacin (lacking cyclopropyl group), react only with fraction A (**Holmes et al, 1985**).

Flumoxine and nalidixic acid were less effective for most isolated bacteria, the decrease in vitro activity of these drugs could be attributed to develop of drug resistance since flumoxine and nalidixic acid are already in veterinary use for many years. In 1962, nalidixic acid was the first quinolone marketed for clinical use. These findings were in consistent with those reported by **Watts et al. (1993) and Rzedzicki et al.(1999)**.

Gentamicin also exhibited good activity against most isolated bacteria, the MICs of gentamicin for *E. coli*, *Salmonella* spp, and *Proteus* spp. in this study were comparable with previously published data (**Saleem et al., 1999 and Salmon and Watts., 2000**).

The isolated strains showed a high prevalence of resistance to amoxicillin, streptomycin, trimethoprim and penicillin specially *Proteus* spp., *Citrobacter* spp. and *Enterobacter* spp. the high prevalence of resistance to these drugs may be related to their use for prophylaxis and control of infectious disease in poultry as well as in the medication of exotic birds. These findings were broadly similar to those recorded (**Bailey et al., 1998 b, Jindal et al., 1999**) in birds.

The results obtained in this study provide information on the gram-negative bacteria isolated from psittacine birds, their drugs susceptibility and the MICs and MBCs. This knowledge improve useful to the clinician when selecting the appropriate antimicrobial agent to treat bacterial infection. Fluoroquinolones undoubtedly have the potential for providing veterinarian with a new arsenal of antimicrobial agents. However, without thoughtful use, the selection of resistant organisms will dramatically reduce the clinical effectiveness of this class of antibacterial agents within a few short years. Pharmacokinetic investigations with fluoroquinolones also warranted to determine dosage regimens in psittacine birds

Table (1): The frequencies of isolation of gram negative bacteria from psittacine birds.

Bacteria	Site of isolation			Total No.	Total %
	Enteric	Respiratory	Organ		
E. coli spp.	3	6	3	12	31.6
Shigella spp.	5	2	1	8	21
Salmonella spp.	5	-	1	6	15.8
Citrobacter spp.	3	1	-	4	10.5
Enterobacter spp.	1	3	-	4	10.5
Proteus spp.	1	1	-	2	5.3
Klebsiella spp.	2	-	-	2	5.3
				38	100%

Table (2): *In vitro* susceptibility of gram-negative bacteria isolated from psittacine birds to commonly used antimicrobial agents by using disc - diffusion method.

Bacterial strains	Mean zones of inhibition (mm) with each respective compound.													
	Enr	Cip	Dif	Nor	Gn	S	Ar	Na	Te	Aml	Do	Cef	P	Trim
E. Coli (12)	23.8 ± 1.7	22.6 ± 1.7	22 ± 1.7	19 ± 1.6	18.2 ± 0.9	15.4 ± 1.7	14.4 ± 1.6	15.9 ± 0.9	11.3 ± 1.6	16 ± 2.12	20.2 ± 1.5	18.5 ± 2.1	9.3 ± 1.9	15.4 ± 3.7
Salmonella (6)	18 ± 0.0	17.5 ± 0.0	15 ± 1.5	14.5 ± 0.2	16.7 ± 1.5	10 ± 1.2	9.3 ± 0.9	9 ± 1.0	11.5 ± 0.9	9 ± 0.6	13.5 ± 0.9	10 ± 2.1	-	-
Kelebsiella (2)	20.3 ± 2.3	19.3 ± 1.7	18 ± 3.2	17 ± 0.5	16 ± 1.5	11 ± 3.0	9 ± 1.5	9 ± 0.0	16.3 ± 2.1	11 ± 0.4	18 ± 0.0	16 ± 3.4	-	-
Shigella (8)	23.7 ± 3.3	20 ± 2.0	20.3 ± 3.8	20 ± 2.3	16.67 ± 1.3	14.8 ± 2.8	17.8 ± 3.0	12 ± 0.0	14.3 ± 4.1	12.3 ± 1.8	20 ± 2.0	17.3 ± 2.3	-	10 ± 2.0
Proteus (2)	19 ± 1.7	19 ± 2.3	15.3 ± 1.5	14.5 ± 0.85	13 ± 1.7	12 ± 0.0	-	-	14 ± 1.7	-	13 ± 1.7	-	-	-
Citrobacter (4)	18 ± 1.8	17 ± 0.6	14 ± 0.8	13 ± 2.4	10.6 ± 2.6	11 ± 0.0	10 ± 0.0	13 ± 1.7	14.3 ± 4.3	14.3 ± 2.0	16 ± 4.0	-	-	9 ± 1.0
Enterobacter (4)	21.3 ± 2.5	19 ± 1.0	18.7 ± 1.8	18.7 ± 1.8	12.8 ± 2.6	10.6 ± 2.3	-	-	12.3 ± 0.9	-	18 ± 1.0	-	-	-

Table (3): Summary of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) (ug/ml) of antimicrobial agents against gram - negative bacteria isolated from psittacine birds.

Bacterial strains	Enrofloxacin		Ciprofloxacin		Difloxacin		Norfloxacin		Gentamicin		Streptomycin		Flamoxone		Mandicic acid		Tetracycline		Amoxicillin	
	MICs	MBCs	MICs	MBCs	MICs	MBCs	MICs	MBCs	MICs	MBCs	MICs	MBCs	MICs	MBCs	MICs	MBCs	MICs	MBCs	MICs	MBCs
E. coli	0.39	0.78	0.78	1.56	0.39	0.78	1.56	3.2	0.78	1.56	3.2	6.25	3.1	6.25	3.1	6.25	12.5	50	6.25	12.5
Salmonella	0.19	0.39	0.19	0.78	0.78	1.56	0.39	1.56	0.39	1.56	15.5	31	3.1	12.5	3.1	6.25	1.5	12.5	0.78	1.56
Kelebsiella	0.19	0.39	0.78	1.56	1.56	1.56	1.56	1.56	0.39	1.56	7.8	15.5	12.5	25	6.25	12.5	1.5	6.25	>100	>100
Shigella	0.39	1.56	0.78	1.56	0.78	1.56	6.25	6.25	0.39	1.56	62.5	>100	3.1	12.5	1.56	6.25	1.5	3.1	0.78	1.56
Proteus	0.39	0.78	0.78	3.1	3.1	12.5	3.2	6.25	6.25	1.56	12.5	12.5	>100	>100	12.5	25	3.1	12.5	62.5	>100
Citrobacter	0.78	1.56	0.78	3.1	1.56	3.1	6.25	12.5	12.5	50	0.39	0.78	>100	>100	1.56	3.1	3.1	12.5	>100	>100
Enterobacter	0.39	0.78	0.78	1.56	1.56	3.1	6.25	12.5	6.25	25	>100	>100	>100	>100	12.5	25	6.25	12.5	>100	>100

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الملخص العربي

تأثير المضادات البكتيرية على بعض البكتريا المعزولة من طيور الزينة
المصابة طبيعياً في محافظة الشرقية

المشركون في البحث

أحلام السيد عبداللطيف و مها عوض الله السيد

أوضحت نتائج الفحص البكتريولوجي واختبارات الحساسية على عدد ٣٨ من الميكروبات السالبة لصبغة الجرام والتي جمعت من ٥٠ طائر من طيور الزينة المختلفة الأنواع والأعمار ما بين مريضة وميتة ومن أماكن مختلفة في محافظة الشرقية، وكانت نسبة عزل الميكروب العصري القولوني ٣١٦٪ وميكروب الشيكلا ٢١٪ والسالمونيلا ١٥٨٪ وكانت نسبة ١٠٥٪ لكل من الستروباكتري والانتريوباكتري كلا من البروتياس والكليسيلا بنسبة ٥٣٪، تم تحديد حساسية هذه الميكروبات المعزولة معملياً لبعض المضادات الحيوية الشائعة الاستخدام وذلك باستخدام اختبار الانتشار خلال الآجار والتخفيف خلال الوسط المغذي، وأثبتت الدراسة أن جميع الميكروبات المعزولة كانت حساسة لكل من الإنترفلوكساسين والسيروفلوكساسين والدايفلوكساسين وكان التركيز الأدنى المثبط للبكتريا يتراوح بين ٠.١٩ : ٠.٣٩، ٠.١٩ : ٠.٧٨، ٠.٣٩ : ١.٥٦ ميكروجرام / مل على التوالي وكان التركيز القاتل للبكتريا مماثل للتركيز المثبط أو ضعفه بينما كانت كفاءة كلاً من النورفلوكساسين والجنتاميسين في المرتبة الثانية وكان أقل تركيز مثبط للبكتريا يتراوح ما بين ٠.٣٩ : ٠.٦٢٥، ٠.٣٩ : ١.٢٥ ميكروجرام / مل، أما بالنسبة لحمض النالديكسك والدوكسي سيكلين والتترايسايكلين والسفتيوفور كانت النتائج متباينة وكانت عترات البروتياس والستروباكتري والإنتروباكتري مقاومة لمعظم المضادات البكتيرية المختبرة ماعدا مركبات الفلوروكينولون والدوكسي سيكلين وكانت معظم العترات المعزولة مقاومة لكل من الأموكساسلين والفلومركوين، والاستريتومايسين والتراميثوبريم والبنسيلين ومن هذه الدراسة تم معرفة مدى إنتشار الميكروبات السالبة لصبغة الجرام في طيور الزينة ومدى حساسيتها للمضادات البكتيرية المتداولة حقيلاً وتحديد أقل تركيز مثبط وأقل تركيز قاتل لهذه الميكروبات، وهذه النتائج تقدم معلومات مفيدة للعاملين في هذا المجال لاختيار المضاد الحيوي المناسب في علاج البكتريا التي تصيب طيور الزينة.