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DETECTION FOR THE MOST SUITABLE ANTIBIOTIC AND DISINFECTANT FOR PSEUDOMONAS AERUGINOSA ISOLATED FROM PNEUMONIC AND APPARENTLY HEALTHY FARM ANIMALS

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ABSTRACT

The present study was carried out on 550 samples (nasal, tracheal swabs and lung tissues) collected from pneumonic and apparently healthy cows and buffaloes. The bacteriological examination revealed that Pseudomonas aeruginosa *was isolated from 172(41.1%) out of 419 cow's samples and from 46(35.1%) out of 131 buffalo's samples. From 172 cow's isolates there were 144 isolates related to serotype (1) and 28 isolates were untypable while buffalo's positive isolates revealed that 40 isolates were belonging to serotype (1) and 6 isolates were untypable. The effect of disinfectants as phenol, formalin and ethyl alcohol on viability of tested strains showed that 3.0% phenol could kill all tested strains after 5 minutes, but 3% concentration of formalin has a marked killing effect on the growth of P. aeruginosa within 10–15 minutes, while 40% ethyl alcohol dilution could kill P. aeruginosa after 10 minute. Forty P. aeruginosa isolates were tested for antibiotic susceptibility to 12 chemotherapeutic agents. The most effective antibiotic was gentamycin (95.0%) then streptomycin (87.5%) and amikacin (75%), all tested strains were completely resistant to tetracycline, cephalothin and ampicillin. It is concluded that P. aerouginosa is one of the most important cause of pneumonia in cows and buffaloes also it resists many disinfectants and remain viable except by using appropriate concentration. In vitro antibiotic sensitivity test showed that most strains were susceptible to gentamycin and amikacin although they resist many types of antibiotics.*

Keywords, *P. aerouginosa, Disinfectant, in vitro antibiotic sensitivity test.*

INTRODUCTION

The effect of respiratory disease is widespread and can be measured as the amount of the direct economic losses happening due to mortality, morbidity, treatment, prevention costs and loss of production (reduced animal performance and carcass quality) and the indirect costs. **MebratuAsaye *et al* (2015) and Jim, K., (2009)**

Bacteria are observed as the most important cause, as primary or secondary pathogens of cattle pneumonia. Predisposing factors play a significant role in exposing the animal to disease **Isam Eldeen Nour Elhuda Elamin (2003).**Different strains of bacteria cause pneumonia such as Pasteurella species (*P. Multocida*), Pseudomonas Sp. and Klebsiella pneumonia **Hamad and Al-Attar (2006).** *P. aeruginosa* is the most common

pathogen involved in nosocomial pneumonia and is responsible for both a high mortality and morbidity in critically ill patients **Fagon JY *et al* (1996).**

The ability of *P. aeruginosa* to invade healthy tissues rarely but when defenses are compromised, it can infect different tissues. This explains why most infections are nosocomial **Mesaros *et al* (2007),** also *P.aeruginosa* can survive on minimal nutritional requirements and tolerate a variety of physical conditions which help this organism to remain viable in both community and hospital settings **Lister *et al* (2009).**

One of the most important features of the bacterium is its resistance to various antibacterial agents and even recently developed antibiotics has failed to reduce the mortality rate associated with this organism **Ali *et al* (2009)**. An increase in the incidence of resistance of *P. aeruginosa* to different chemotherapeutic agents has been described from many parts of the world and may be due to pigment production and to the large increase of therapeutic use of antibiotics between different domestic animals. **Balakrishnan *et al* (2004)**.

P. aeruginosa characterized by inherent resistances to a wide variety of antimicrobials. Its intrinsic resistance to many antimicrobial agents and its ability to develop multidrug resistance beside mutational acquired resistance to antibiotics through chromosomal mutations makes a serious therapeutic problem **Zahraa *et al* (2014) and Al-Grawi (2011).** Therefore, this study was designed to detect the effective concentration of the most common used disinfectant and also the most suitable antibiotics which help in control of *P. aeruginosa*.

MATERIAL AND METHOD

Samples

During this study a total of 550 samples were collected from pneumonic and apparently normal cows and buffaloes as described in Table (1). All samples were obtained from different private farms and abattoirs in Dakahlia Governorate and slaughter house in Basateen.

Bacteriological examination.

An inoculum from broth containing the sample was cultivated onto the following media in duplicate including: Pseudomonas agar base with C-N (cetrimidenalidixic acid) supplement, blood agar, MacConkey's agar and nutrient agar plates. All inoculated plates were incubated aerobically at 37^{o}C for 24-48 hrs. then examined. Suspected colonies were described for their appearance, hemolytic activity, and colonial characters according to **Konema*et al*. (1992).**

Biochemical examination: (MacFaddin, 1980): Such as oxidase test**,** motility test**,** sugar fermentation test, nitrate reduction test, urease activity test**,** aesculin test.

Serological identification of the isolates: Unheated viable cells were used as an antigen after adding droplets of Bacto- *P. aeruginosa* antisera (diluted 1:10) on the appropriate squares of the glass plate then mix the isolated colony with the droplet of antisera. Finally, the plate was rotated by hand for about a minute and then observed for the presence of agglutination.

Effect of certain chemical agents on *P. aeruginosa:* The influence of certain disinfectants on *P. aeruginosa* were carried out by using the suspension test concentration /

time relationship according to the method described by **(Block *et al*.,1977).** Common disinfectants namely: phenol, ethyl alcohol, formalin were examined to detect their action on *P. aeruginosa*. **Antibiotic sensitivity for the isolated *P. aeruginosa*:** The test diffusion technique was applied according to **(Finegold and Martin, 1982**).

RESULT

Incidence of *P. aeruginosa* obtained from examined diseased and apparently normal cows and buffaloes: As shown in table (3) among a total of 419 cows samples (nasal, tracheal and lung) only172(41.1%) were positive for *P. aeruginosa* while the result of buffaloes samples showed that out of 131 samples (nasal, tracheal and lung) only 46(35.1%) were positive for *P. aeruginos*.

Prevalence of *P. aeruginosa* isolates recorded from apparently normal and diseased cows and buffaloes: As regards to bacteriologically positive cases, the results showed that 37 samples out of 95 samples and 14 sample out of 34 sample collected from apparently normal cows and buffaloes were positive for *P. aeruginosa* with incidence of 38.95% and41.17% respectively, on the other hand 135 samples out of 324 and 32 samples out of 97 samples collected from respiratory affected cows and buffaloes were positive for *P. aeruginosa* with a percentage of isolation reached 41.7% and 32.9% respectively, as shown in table (4).

The results of serological identification of isolated strains of *P. aeruginosa* from cows showed that 144 isolates were belong to serotype (1) and 28 isolates were untypable and the results of serological identification of isolated strains of *P. aeruginosa* from buffaloes showed that 40 isolates were belong to serotype (1) and 6 isolates were untypable.

Effect of some disinfectants on the viability of *P. aeruginosa*:

Effect of phenol solution on viability of *P. aeruginosa:* Laboratory studies on the effect of phenol on the viability of *P. aeruginosa* revealed that *P. aeruginosa* were able to resist 1.0% phenol and remained viable for a period of 20-25 minutes. Also it is able to resist 2.0% phenol and remained viable for a period of 15-20 minutes. Moreover, 3.0% phenol could kill all tested strains after 5 minutes and 5.0% phenol solution could destroy tested *P. aeruginosa* immediately, as shown in table (6).

2. Effect of formalin solution on the viability of *P. aeruginosa*: Studying the effect of formalin on the viability of *P. aeruginosa* revealed that 1% formalin solution inhibit the growth of the organism after 25 minutes, but 3% concentration of formalin had a marked killing effect on the growth of *P. aeruginosa* within 10–15 minutes. Concentration of 7.0% have powerful bactericidal effect immediately on the examined *P. aeruginosa*, as show in table (7).

3. Effect of ethyl alcohol solution on the viability of *P. aeruginosa*: Studying the results of the effect of different concentrations of ethyl alcohol on the viability of *P. aeruginosa* revealed that 30% ethyl alcohol could succeed to destroy *P. aeruginosa* within 30 minutes, while 40% dilution could killed *P. aeruginosa* after 10 minutes. Meanwhile, as the concentration increase, the time required was respectively decreased since at a concentration of 60.0% the bactericidal action appeared after 2.5 minutes, as shown in table (8).

Results of antibiotic sensitivity test of *P. aeruginosa*:The in vitro sensitivity of 40 *P. aeruginosa* isolates from cows &buffaloes

were done against 12 chemotherapeutic agents. Reviewing results of cows and buffalo strains, the most effective antibiotic was gentamycin (95.0%) and streptomycin (87.5%), these were followed by amikacin (75.0%). Also, polymxin B, Neomycin, chloramphenicol and flumequine could be considered in another category and their affect ranged between 15.0 – 50.0% all tested strains were completely resistant to tetracycline, cephalothin and ampicillin, as shown in table (9).

Table (1): Number and types of examined samples

Animal species	Type of samples	Apparently normal	Diseased	Total
Cows	Nasal swab	65	174	**239**
	Tracheal swab	10	30	**40**
	Lung tissues.	20	120	**140**
Total		**95**	**324**	**419**
Buffaloes	Nasalswabs.	20	67	**87**
	Trachealswabs.	9	13	**22**
	Lung tissues.	5	17	**22**
Total		**34**	**97**	**131**
Overall total		**129**	**421**	**550**

Table (2): Interpretation of zones of inhibition on agar diffusion method for antibacterial susceptibility. According to (Oxide)

Antimicrobial agent	Discontent	Diameter of inhibition		
		Resistance	Intermediate	Sensitive
Amikacin	30 µg	14 or less	14 – 16	**17 or more**
Ampicillin	10 µg	11 or less	12 – 13	**14 or more**
Cephalothin	30 µg	14 or less	15 – 17	**18 or more**
Chloramphenicol	30 µg	12 or less	13 – 17	**18 or more**
Erythrocin	15 µg	13 or less	14 – 17	**18 or more**
Flumequine	30 µg	13 or less	14 – 15	**16 or more**
Gentamicin	10 µg	12 or less	13 – 14	**15 or more**
Neomycin	30 µg	12 or less	13 – 16	**17 or more**
Polymyxin B.	30 µg	12 or less	12 – 13	**13 or more**
Streptomycin	10 µg	11 or less	12 – 14	**15 or more**
Tetracycline	30 µg	14 or less	15 – 18	**19 or more**
Trimethoprim sulphamethoxazole	**1.25 + 5.75**	**10 or less**	**11 – 15**	**16 or more**

Table (3): Incidence of *P. aeruginosa* obtained from examined diseased and apparently normal cows and buffaloes

Animal	Type of Samples	Total examined samples	Diseased			Apparently normal		
			No. of examined samples	No. (+ve)	%	No. of examined samples	No. (+ve)	%
Cows	Nasal swab	239	174	65	37.35	65	24	**36.9**
	Tracheal swab	40	30	11	36.6	10	4	**40**
	Lung	140	120	59	49.2	20	9	**45**
	Total	**419**	**324**	**135**	**41.7**	**95**	**37**	**38.95**
Buffaloes	Nasal swab	87	67	25	37.3	20	9	**45**
	Tracheal swab	22	13	2	15.4	9	1	**11.1**
	Lung	22	17	5	29.4	5	4	**8**
	Total	**131**	**97**	**32**	**32.9**	**34**	**14**	**41.17**

Table (4): Prevalence of *P. aeruginosa* isolates recorded from apparently normal and diseased cows and buffaloes.

Animal status	No. of bacteriologically examined samples	Positive	
		No.	%
App. Normal cows	95	37	38.95
Diseased cows	324	135	41.7
Total	419	172	41
App.Healthy buffalo	34	14	41.17
Diseased buffaloes	97	32	32.9
Total	131	46	35.1

Table (5): Results of serotyping of *P. aeruginosa*.

Animals	Total positive strains	Type1	Untypable
Cows	172	144	28
Buffaloes	46	40	6

Table (6): Effect of phenol on the viability of *P. aeruginosa*.

Time / minute of exposure	Phenol concentration			
	1%	2%	3%	5%
0.5 min	+	+	+	-
2.5 min	+	+	+	-
5	+	+	+	-
10	+	+	-	-
15	+	+	-	-
20	+	+	-	-
25	+	-	-	-
30	-	-	-	-

(+) = Resist **(-) = kill all tested strain**

Table (7): Effect of formalin solution on *P. aeruginosa* viability.

Time / minute of exposure	Formalin concentration			
	1%	3%	5%	7%
0.5 min	+	+	+	-
2.5 min	+	+	+	-
5	+	+	+	-
10	+	+	-	-
15	+	+	-	-
20	+	-	-	-
25	+	-	-	-
30	-	-	-	-

(+) =resist **(-) = kill all tested strains.**

Table (8): Effect of different concentration of ethyl alcohol on *P.aeruginosa*.

Time / minute of exposure	Ethylalcohol concentration			
	30%	40%	50%	60%
0.5 min	+	+	+	+
2.5 min	+	+	+	+
5	+	+	+	-
10	+	+	-	-
15	+	-	-	-
20	+	-	-	-
25	+	-	-	-
30	-	-	-	-

(+) =resist **(-) =kill all tested strain.**

Table (9): Results of antibiotic sensitivity test of *P. aeruginosa*.

Antibacterial agents	*P. aeruginosa* strains (40)			
	Sensitive		Resistant	
	No.	%	No.	%
Amikacin	30	75	10	**25**
Ampicillin	0	0.0	40	**100.0**
Cephalothin	0	0.0	40	**100.0**
Chloramphenicol	10	25	30	**75**
Erythrocin	1	2.5	39	**97.5**
Flumequine	3	7.5	37	**92.5**
Gentamycin	38	95	2	**5**
Neomycin	8	20	32	**80**
Poly myxin. B.	20	50	20	**50**
Streptomycin	35	87.5	5	**12.5**
Tetracycline	0	0.0	40	**100.0**
Trimethoprim	1	2.5	39	**97.5**
Sulphamethoxazole	**0**	**0.0**	**40**	**100.0**

DISCUSSION

Respiratory tract infections are a common manifestation in ruminant species of animals. It is considered that the bacterial flora of the respiratory system includes both inhabitant and temporary **Quinn, P.J.*et al* (2002),** *Pseudomonas aeruginosais*, a Gram-negative bacterium with an extraordinary physiological and metabolic adaptability. This organism has a widespread in nature Moreover, it tolerates a variety of physical conditions, and is able to persist in both public and hospital settings **Francisco Toval *et al.* (2014) and Silby *et al*., (2011).**

In veterinary medicine, this bacterial species is increasingly noted as the cause of severe infection among domestic animals and birds. In cattle, *P. aeruginosa* is the common cause of respiratory affections and mastitis, cutaneous abscesses, ocular lesions, genital tract infections and incriminated as a cause of seminal vesiculitis (**Radchenkov *et al*., 1993).** Thus, particular attention has been directed towards *P. aeruginosa* due to its gradual emergence as some significant animals, birds as well as human pathogens, few reports dealing with the comparative studies on various strains of *P. aeruginosa* isolated from cattle and buffaloes in Egypt, so the questions about their incidence, serotyping are still in question. A variety of cultural media have been applied in the present work to improve the isolation, then complete identification of the isolates from examined different clinical samples and apparently normal sample, in order not to miss the isolates. All isolated strains belonging to *P. aeruginosa* were extensively studied for their morphological, cultural, biochemical and serological characteristics were done.

It is worthy to note that the incidence of *P. aeruginosa* between examined cattle and buffaloes were 41.1% and 35.1% respectively as shown in table (3). These findings go hand with the observations of **Rajasekhr *et al*., (1992) and ManalBahaa (2004)** who isolated *P.aeruginosa* from cows in higher incidence (15.4%) than in buffaloes (10.5%).

The results given in table (4) revealed that *P. aeruginosa* were isolated from apparently healthy and diseased cows in an incidence of 38.9% and 41.7% respectively while from buffaloes in an incidence of 41.1% and 32.9% respectively. These findings tend to agree with **Ackermann *et al* (1996) and ManalBahaa (2004)** who isolated 68 strains of *P. aeruginosa* from the intestinal and respiratory systems of apparently normal cattle and102 strains of *P. aeruginosa* from respiratory tract, genital tract, wound secretions from diseased cattle suffering from pneumonia, endometritis and wound affections in an incidence of 14.8%. In table (5) serological typing of 172 *P. aeruginosa* strains isolated from cows, 144 strains were belonging to serotype (1) and 28 strains were untypable with the available antisera and concerning serological identification of 46 strains of *P. aeruginosa* isolated from buffaloes, 40 strains were belonging to serotype 1 and 6 strains were untypable with available antisera. Similar findings were reported by **Riad (1994)** differentiated *P. aeruginosa* strains into nine serotypes and untypable groups and the most prevalent was serotype "1" (34.1%) and 2 strains were untypable serologically. On the other hand, **He *et al,* (1998)** isolated different serological type from 101 strains of *P. aeruginosa* with the typed rate of 84.2%. Among them type (7) 23.7%, type (1) 13.9% and another strains 15.8% could not be differentiated and so named untyped *P. aeruginosa* group.

The present work was carried with the aim to determine the effect of several disinfectant and antibacterial agents on the viability of *P. aeruginosa*. In fact, there are Scanty data in the literatures on the effect of the disinfectants on *P. aeruginosa* was recorded, so the results in the present work will be discussed according to the effect of the most common used disinfectants namely: phenol, formalin and ethyl alcohol on *P. aeruginosa* isolated from cows and buffaloes. Past studies on the response of *P. aeruginosa* to disinfectants have shown certain discrepancies of *P. aeruginosa* to disinfectants **Lowbury (1951).** As shown in table (6) 1-2% phenol solution failed to eliminate *P. aeruginosa* before 20-25 minutes' exposure, while higher concentrations as 3% - 5% has a bactericidal effect after variable periods of time. It was noticed that all tested strains of *P. aeruginosa* required a comparatively nearly similar time for their death, although the difference in the time factor did not exceed 5 minutes or lesser. Generally, the value of phenol as a disinfectant is obvious. Nearly similar results were reported by **Oryan *et al.*, (2007) and Korenova (2008)** who recorded that phenol and many phenolic compounds are strong antibacterial agents especially at the generally employed concentrations (1-3% aqueous solutions) due to denaturation of the vital protein. As shown in table (7), it is noticed that 1% formalin is considered highly effective against *P. aeruginosa*in 25- 30 minutes. On the contrary at the concentration of 7% solution destroyed all tested strains at once. Nearly similar results were reported by **Amany El Gohary (2004) and Rani Abd El wahab (2009).** As shown in table (8), ethyl alcohol in high concentration of 60.0% is excellent antibacterial agents *P. aeruginosa*within 2.5mm. Up to 5 minutes' post exposure. Ethyl alcohol is commonly used as disinfectants because it is toxic to bacterial cells due to the denaturation of protein. **Oryan *et al.*, (2007) and Korenova (2008).**

Most of isolated strains proved to be highly sensitive to gentamicin (95%), amikacin (75%) and streptomycin (87.5%). There was little or no sensitivity to neomycin, flumequine, chloramplenicol, tetracycline, erythromycin, cephalothin and trimethoprinsulfamethoxzole (table 9). Aminoglycosides including gentamicin was the most effective inhibitors of growth and was used here as the representative of the class of antibiotics together with amikacin as the best of choice for infections due to *P. aeruginosa*. These results are in agreement with that of **Akhoon Z.A *et al* (2012)** who reported that the most effective antibiotics on *P.* aeruginosa were gentamycin and amikacin. Meanwhile, **Omae *et al.*, (1974)** isolated 118 strains of *P. aeruginosa*, from clinical specimens from cattle, equines and pigs and found that all tested strains showed a high sensitivity to colistin, polymyxin and gentamycin and all strains were resistant to kanamycin, erythromycin, oleandomycin, chloramphenicol and sulpha drugs. Similar observations were also registered by many workers **Longford *et al.*, (1990) and Riad (1994).** They reported the sensitivity of amikacin and gentamicin against *P. aeruginosa*. Also the same results reported by **Tre-Hardy *et al.*, (2008) and Rania Abd El Wahab (2009).** The isolates are remarkable for their intrinsic lack of susceptibility to many antimicrobial agents. In general, most workers agree that most of antibiotics are of limited value in the treatment of *P. aeruginosa* infection in animals.

The need for sensitivity testing is limited in large animal practice, because the results frequently come too late to be useful, but it is beneficial for prophylaxis program. However, talking samples may be important to confirm the initial choice of therapy. Thus, sensitivity testing is intended to give a basis for the choice of an antimicrobial drug.

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الملخص العربي

تحديد لاهم انواع المضادات الحيويه والمطهرات المؤثره علي السيدموناس اريجينوزا المعزوله من حيونات المزرعه المصابه بالالتهاب الرئوي واخري تبدو سليمه ظاهريا

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في هذه الدراسة تم الفحص البكتريولوجي علي عدد ٥٥٠ عينة من الأبقارو الجاموس هذه العينات تم جمعها من حيوانات تبدوا سليمة ظاهريا وأخري تظهر عليها أعراض الالتهاب الرئوي وهذه العينات تم فحصها للحصول علي صورة كاملة لميكروب السودوموناس اريجينوزا سواء كان الحيوان يبدو سليما ظاهريا أو تظهر عليه أعراض الالتهاب الرئوي.

أثبت الفحص البكتريولوجي أن هناك ١٧٢ عينة ايجابية من أصل ٤١٩ عينة تم جمعها من الأبقار بنسبة ٤١% سواء كانت هذه الأبقار تبدوا سليمة ظاهرياً أو تبدوا عليها أعراض الالتهاب الرئوي أما بالنسبة للعينات التي تم جمعها من الجاموس فكان هناك ٤٦ عينة إيجابية من أصل ١٣١ بنسبة ٣٥,١% .تم عمل التصنيف السيرولوجي للعينات الايجابيه فوجد ان ١٤٤ و٤٠ عينه من ١٧٢ و٤٦ عينه تم عزلها من الابقار والجاموس علي التوالي تنتمي للنوع السيرولوجي (١) و بدراسة مدي تاثير بعض المطهرات عليها مثل الفينول والفورمالين وكذلك الكحول الايثيلي وجد ان الفينول بتركيز ٣% قادر علي القضاء السيدموناس اريجينوزا نهائيا بعد ٥ دقائق بينما الفورمالين له تاثير قوي علي السيدموناس اريجينوزا في خلال ١٥ دقيقه كما وجد ان تركيز ٤٠% من الكحول الايثيلي قادر علي القضاء علي السيدموناس اريجينوزا بعد ١٠ دقائق علي الاقل اما بالنسبة لحساسية المعزولات للمضادات الحيوية وجد أن الجينتاميسين والإستربتومايسين وكذلك الاميكاسينمن اهم المضادات الحيويه التي لها تأثير قوي في علاج السيدوموناس اريجينوزا.