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APPLICATION OF ELECTEROPHORESIS AND IMMUNOBLOT IN DIAGNOSIS OF PSEUDOMONAS AERUGINOSA ISOLATED FROM RESPIRATORY SYSTEM OF CAMELS

(With 4 Tables and 2 Figures)

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تطبيق اختبار الفصل الكهربائى والطبع المناعى فى تشخيص ميكروب الصديد الاخضر المعزول من الجهاز التنفسى للجمال

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اجريت هذه الدراسة على عدد 70 جمل مذبوح فى المجازر وأشتملت الدراسة على 210 عينة (70 عينة من كل من مسحات من القصبة الهوائية ، الرئة والغدد الليمفاوية) لفحصها بكتريولوجياً ولقد تم عزل ميكروب الصديد الاخضر بنسبة 4,28% ، 8,57%، 4,28% على التوالى وتم اجراء اختبار الحساسية لكل من العترات البكتيرية المعزولة ولقد وجد ان معظم العترات البكتيرية المعزولة شديدة الحساسية لكل من الجنتاميسين والكلورامفنيكول ومقاومة لكل من الاماكين والفلوكاسيللين والبنسللين والنيوميسين والأمبسللين. وقد تم اجراء اختبار الفصل الكهربائى واختبار الطبع المناعى للميكروب وقد اثبت اختبار الفصل الكهربائى ان كسور البروتين لميكروب الصديد الاخضر تتروح بين 62,123 كيلو دالتون الى 18,8 كيلو دالتون كما اثبت اختبار الطبع المناعى ان الاجسام المضادة احادية المنشا الخاصة بالغشاء الخارجى لميكروب الصديد الاخضر تتفاعل مع حزمة البروتين عند الوزن الجزيئ 28017 كيلو دالتون بينما حزم البروتين الخفيفة كانت عند الوزن الجزيئ 61,203 كيلو دالتون، 46,148 كيلو دالتون، 35,024 كيلو دالتون، 8,1954 كيلو دالتون

SUMMARY

This Study was carried out on 70 slaughtered camels. 210 samples (70 each of tracheal swabs, lungs and lymph nodes) for isolation of *Pseudomonas aeruginosa*. Obtained results declared that *Pseudomonas aeruginosa* could be detected in 3 (4.28%), 6 (4.28%) and 3 (4.28%) of the examined samples of tracheal swabs, lungs and lymph nodes respectively. The in vitro antibiotic sensitivity tests for the isolated

bacteria were discussed. The results showed that *Pseudomonas aeruginosa* was sensitive to Gentamycin and Chloramephenicol, while this organism was resistant to Amikacin, Flucloxacillin, Penicillin, Neomycin and Ampicillin. SDS- PAGE demonstrated that the protein fractions of *Pseudomonas aeruginosa* ranged between 62.123 KDa and 18.8 KDa. Immunoblotting analysis of the OMPs of *Pseudomonas aeruginosa* demonstrated that the monoclonal antibody specific to *Pseudomonas aeruginosa* OMPs reacted with protein band at molecular weight of 28.017 KDa (more immunogenic band) while faint bands were revealed at 61.203 KDa, 46.148 KDa and 8.1954 KDa.

Key words: *Camels, respiratory system, Pseudomonas aeruginosa.*

INTRODUCTION

Camel is an animal of considerable importance in Egypt since it is one of the major sources of meat production where its meat represents 66.46 % of total meat obtained from the imported animals for slaughter purposes (Anon, 1986). Also the camel plays vital socioeconomic roles and supports the survival of millions of people in Asia and Africa. It is being used as a source of protein, milk, hide as well as quite and effective mean of transport (Chowdhary, 1986).

Respiratory diseases of camels continue to be a major cause of economic loss and adverse on animal. Stress of cold weather, rain, bad hygiene and high humidity rate were incriminated to increase the respiratory infection. Respiratory affection is the main cause of death among camel calves allover the world (Chowdhary, 1986; Khanna *et al.*, 1992).

Several species of organisms could be isolated from both apparently healthy and affected respiratory tract of camel, *Pseudomonas aeruginosa* is one of them (Amany, 2000; Fatma *et al.*, 2001; Seddak, 2002).

Electrophoretic analysis and immunoblotting are considered one of the most advanced techniques used for characterization of antigens (Laemmli, 1970; Towbin *et al.*, 1979).

Hence, the present work aimed to isolate *Pseudomonas aeruginosa* from respiratory system of camels, in-vitro antibiotic sensitivity against the isolated strains and identification of *Pseudomonas aeruginosa* by Sodium dodecyl sulfate / polyacrylamid gel electrophoresis (SDS-PAGE) and Immunoblotting

MATERIALS and METHODS

1- Sampling:

A total of 210 samples including 70 each of tracheal swabs, lungs and thoracic lymph nodes tissues were collected under aseptic condition from 70 slaughtered camels in different abattoirs. All samples were transported as quickly as possible to the laboratory in an ice box for bacteriological examination.

2- Bacteriological Examination:

Each sample was cultured into cetrimide broth and incubated at 42°C for 48 hours. A loopful was taken from the enrichment broth and cultured onto cetrimide agar (Oxoid. M3) and Pseudomonas Agar f base (King *et al.,* 1954). After incubation the presence of *Pseudomonas aeruginosa* was detected by blue green pigment production (soluble in both water and chloroform) and the cultures have a distinctive smell (fruity smell) on the media used due to 2 amino acetophenone production. From the suspected colonies, agar slants were made and incubated for further identification according to king *et al.* (1954) and incubated at 42°C for 48 hours.

3- In-vitro antibiotic sensitivity test:

The disc diffusion technique was performed on the isolated bacteria from infected cases according to Finegold and Martin (1982) using Norfloxacin, Cefoperazone, Amikacin, Flucloxacillin, Penicillin, Neomycin, Ampicillin, Gentamycin and Chloramephenicol. The degree of sensitivity was determined and interpreted according to Oxoid (1998).

4- Preparation of outer membrane protein antigen (OMPs)

Each of the strains was cultivated in 500 ml of broth medium for 2 days at 37°C, the cells were sedminted by centrifugation at 13,000XG for 20 minutes, washed twice in 0.25 M NaCl and resuspended in 1.5 ml of M NaCl. One ml of cell suspension was combined with 2 ml of phenol- acetic acid- water (2:1:0.5 w/v/v) then after 3 to 4 hours at 25°C, the insoluble material was sedimented by centrifugation at 30,000XG for 15 minutes and the supernatant fluid was decanted and maintained at5°C until used (Dubreuil *et al.,* 1988).

5- Electrophoresis for OMPs

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE) of the OMPs samples of *Pseudomonas aeruginosa* isolates were carried out as described by Laemmli (1970) using pre-stained high molecular weight standard marker (Sigma Chemical

company St. Louis Mo 63178 USA. The bands of mol.w. protein marker from top to bottom are: 66. KDa, 45 KDa, 34.7 KDa, 24 KDa, 18.4 KDa and 14.3 KDa.

After electrophoresis, the gel was stained by coomassi blue stain and distained according to Hitchock and Brown (1983). Dendogram was constructed for similarity with each isolates (Advanced American Biotechnology, UPGMA, USA).

6- Imunblotting (Western blot) according to Towbin *et al.* (1979):

After electrophoresis the gels were transferred onto nitrocellulose membrane. The membrane were incubated with rabbit anti-bovine Ig G conjugated with HRP (Horse raddish peroidase), using Biolab - broad rang pre- stained Molecular weight marker.

The color was developed by adding the substrate (30mg 4-chloro – 1–naphthol dissolved, 10 ml cold methanol, 30 µl hydrogen peroxide in 50 ml PBS pH 7.4). At the end of transfer process, protein bands were checked on nitrocellulose membrane by staining with 1 % amido black stain.

RESULTS

Table 1: Incidence of *Pseudomonas aeruginosa* isolated from the respiratory tract of camels samples.

Types of samples	No. examined	Positive samples	
		No.	%
Tracheal Swabs	70	3	4.3%
Lungs	70	6	8.6%
Lymph nodes	70	3	4.3%
Total	210	12	5.7%

Table 2: Antibiotic sensitivity of the isolated *Pseudomonas aeruginosa* from the respiratory tract of camels.

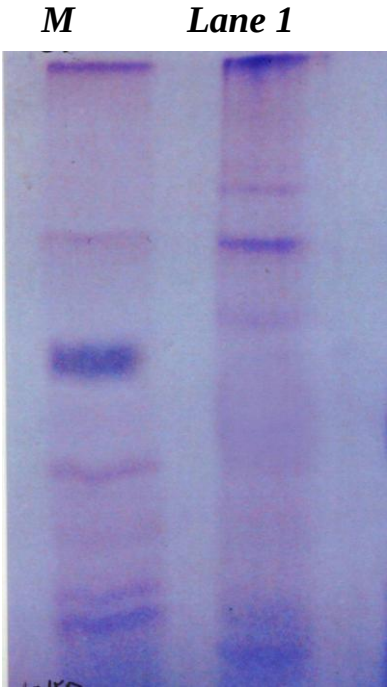
Antibiotic and chemotherapeutic agents	Conc.	Symbol	Reaction
Norfloxacin	10 ug	NOR	M
Cefoperazone	75 ug	CFP	M
Amikacin	30 ug	AK	R
Flucloxacillin	5 ug	FL	R
Penicillin	10 ug	P	R
Neomycin	30 ug	N	R
Ampicillin	30 ug	AM	R
Gentamycin	10 ug	GM	S
Chloramephenicol	30 ug	C	S

S= sensitive
M= Moderate sensitivity R= Resistant

Table 3: The molecular weight (mol. w.) of electrophoresis of *Pseudomonas aeruginosa* compared with the mol.w. of the marker.

Lanes Rows	Marker (mol. W.)	Lane 1 (mol. W.)
R1	66	62.123
R2	45	41.158
R3	34.7	39.158
R4	24	13.631
R5	18.4	18.8
R6	14.3	

Photo 1: Electrophoretic profile of Pseudomonas aeruginosa isolate detected by SDS-PAGE with coomassie stained



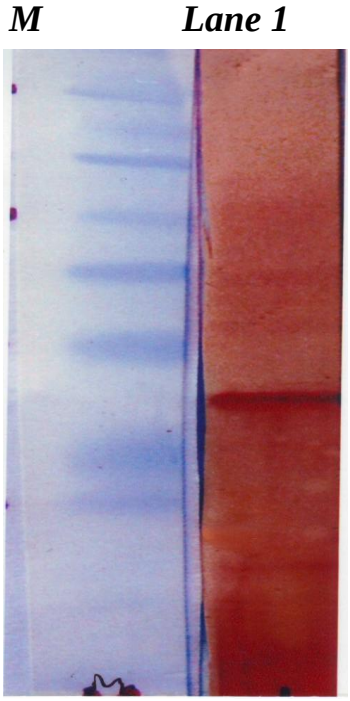
M: Molecular weight marker (Sigma chemical company st. Louis Mo 63178 USA. the bands of mol.w. protein marker from top to bottom are: 66. KDa, 45 KDa, 34.7 KDa, 24 KDa, 18.4 KDa and 14.3 KDa)

M = Marker
Lane 1= Pseudomonas aeruginosa
KDa = Kilodalton.

Table 4: The molecular weight (mol. w.) of western blot of *Pseudomonas aeruginosa* compared with the mol.w. of marker.

Lanes Rows	Marker (mol. W.)	Lane 1 (mol. W.)
R1	175	61.203
R2	83	46.148
R3	62	35.024
R4	47.5	28.017
R5	32.5	8.195
R6	25	
R7	16.5	

Photo 2: Immunoblot of SDS-PAGE of outer membrane protein extracted from and transferred onto nitrocellulose paper recognized by rabbit antiserum, detected with HRP (Horse raddish peroidase) and visualized with color substrate.



M: Molecular weight marker (Biolab - broad rang pre- stained Molecular weight marker)

M = Marker.
Lane 1= Pseudomonas aeruginosa.
KDa = Kilodalton.

DISCUSSION

Bacterial infection of the respiratory tract of camels represents important problems confronting animal production. The present study deals with the pathogenic bacteria in the respiratory tract of slaughtered camels (Amany, 2000).

The results recorded in Table 1 showed that bacteriological examination of 70 tracheal swab samples, 70 lung samples and 70 lymph node samples collected from 70 slaughtered camels revealed that 3 (4.28%), 6 (8.57%) and 3 (4.28 %) respectively were positive for

Pseudomonas aeruginosa (Buxton and Fraser, 1977), also these results nearly agree with those of Fatma *et al.* (2001).

In vitro, the susceptibility of the isolated *Pseudomonas aeruginosa* to different antibiotics is represented in Table 2; the isolated bacteria was highly sensitive to Gentamycin and Chloramephenicol while was resistant to Amikacin, Flucloxacillin, Penicillin, Neomycin and Ampicillin. These findings are partially in agreement with those mentioned with Riad (1989); Abd El-kader (1992); Thabet (1993); Ahmed (1994); Seddak (2002).

The resistance of bacterial isolates to some antibiotics may be attributed to wrong dosage, duration of treatment and route of administration (Amstutz and Armostrong, 1982).

SDS- PAGE is considered as a powerful tool to differentiate genetically related microorganisms (Wassenaar and Newell, 2000)

Electrophoresis of protein was carried out in polyacrylamide gel (10%) where protein was dissociated into their individual polypeptide subunits. This method was discussed by (Laemmli, 1970; Gardon, 1980; Smith, 1984).

In order to identify the electrophoretic profile of the isolated *Pseudomonas aeruginosa*, the protein components were separated by SDS- PAGE and resolved into protein bands as shown in Photo (1) and Table 3. The protein fractions of *Pseudomonas aeruginosa* ranged between 62.123 KDa and 18.8 KDa. The result is nearly similar to that obtained by Doring *et al.* (1987); Pablo *et al.* (1989); Jyotsna *et al.* (2005); Jennifer *et al.* (2009).

Immunoblotting analysis of the OMPs of *Pseudomonas aeruginosa* demonstrated that the monoclonal antibody specific to *Pseudomonas aeruginosa* OMPs reacted with protein band at molecular weight of 28.017 KDa (more immunogenic band) while faint bands were revealed at 61.203 KDa, 46.148 KDa and 8.1954 KDa as shown in photo (2) and Table (4). This result was nearly similar to that obtained by (Pablo *et al.,* 1989; Molakala *et al.,* 1992; Jennifer *et al.,* 2009).

Finally, more efforts must be done to overcome respiratory disorders problem. Periodical clinical and bacteriological examination of apparently healthy animals should be done to avoid misuse of antibiotics. Adequate hygienic measures and proper management of animals would reduce the degree of exposure of animals to disease producing agents. SDS- PAGE and Immunoblotting analysis may be considered as a rapid, reliable, sensitive and diagnostic method to characterize *Pseudomonas aeruginosa*.

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