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Frequency and Antimicrobial resistance of bacteria isolated from oral and topical medicaments from Hilla, Iraq

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Abstract:

Introduction: The presence of microorganisms in pharmaceuticals is undesirable because they may cause spoilage of the product and may present an infection hazard to the consumers or patients.

Methodology: A total of 102 samples of oral and topical non-sterile pharmaceutical products were collected at random from different specialties that are available in Iraq, to investigate the microbial contamination of these products. Bacterial isolates recovered from these medicaments were subjected to susceptibility testing against various antibiotics by disk diffusion method according to Clinical and Laboratory Standards (CLSI) guidelines.

Results: The results revealed that the occurrence of Gram-positive bacteria was in oral and topical medicaments while Gram-negative bacteria were only detected in topical medicaments. More than 58 % of *Bacillus* isolates were resistant to Lincomycin and *Bacillus mycoides* isolates were resistant to ß-lactams and trimethoprim-sulfamethoxazole. *Staphylococcus* spp. showed a relatively high resistance to Ampicillin, Amoxicillin, Penicillin, Tetracycline, and SXT. *S. epidermidis* had the highest number of multi- resistant isolates. 87.5% of isolated Gram-negative rods showed high resistance to ß-lactam antibiotics and 75 % of them were highly resistant to Erythromycin. One isolates of *Pseudomonas aeruginosa* was the most resistant among them.

Conclusion: The high rate of resistance to antimicrobial agents of bacterial isolates recovered from oral and topical medicaments in this study may indicate a widespread antibiotic resistance among bacteria isolated from different sources, including that of anthropological and environmental origin.

Keywords: Antibiotic resistance, bacteria, contamination, medicaments, *Pseudomonas*

INTRODUCTION:

Several papers have been published and reported contamination of non-sterile pharmaceuticals with microorganisms (1, 2 , 3). The presence of microorganisms in pharmaceuticals is undesirable because they may cause spoilage of the product and may present an infection hazard to the consumers or patients.

Several cases of infections caused by the administration of non-sterile medicaments with micro-organisms have been reported (4 ,5). The probability of infection depends on a number of factors including the type of microorganism, the numbers present, the route of infection, and the health status of the host (6).

According to these findings, many developed countries have adopted microbiological standards as well as the application of good manufacturing practices in order to guarantee the hygienic quality of the non-sterile medicaments (7 , 8).

The number of bacterial strains capable of causing infections is increasing, and many of them are resistant to one or more of the antibiotics used in therapy and this resistance constitute an increasingly serious threat to the current antimicrobial agents therapy (9).

The acquisition of resistance to the antimicrobial agents may be due to chromosomal mutation or plasmids that are capable of transfer from one strain of bacteria to another, even across the species barrier. Also the resistance genes are found on mobilizable genetic elements called transposons (10).

Some transposons or plasmids have genetic elements termed integrons that enable them to capture exogenous genes. A number of genes may therefore be inserted into a given integron , resulting in resistance to multiple antimicrobial drugs (11).

The importance of resistant environmental strains is that in certain favorable conditions they may transfer their resistant plasmids to the pathogenic strains. This problem is very serious especially in hospitals where the environment can be a factor for the selection of multiple drug resistant strains (10). If these resistant bacteria are present in the medicaments they may behave as opportunistic pathogens and initiate an infection, especially in immunocompromised patients.

The aim of this study was to investigate the microbial contamination of some non-sterile pharmaceuticals present in Iraq and study the susceptibility to selected antibiotics of bacterial isolates recovered from these medicaments.

MATERIALS AND METHODS:

Medicaments samples:

A total of 102 samples of oral and topical non-sterile pharmaceutical products were collected at random from different specialities that are available in Iraq except those which included antimicrobial

agents and some syrups. Topical medicaments included: 11 ointments, 2 creams, 2 lotions, and 2 pastes .Oral medicaments included: 38 tablets, 8 pills, 16 capsules, 9 powders, 12 syrups, and 2 solutions .All the medicaments were registered trade mark specialties.

To prepare the sample, 1 gram or 1ml each from two different containers was taken and mixed. These mixtures were then resuspended in 90 ml of normal saline and decimal solutions were prepared. Oral medicaments were diluted in phosphate buffer solution.

Bacterial isolates and susceptibility tests:

Aerobic viable bacteria were isolated by plating of decimal dilutions on the following culture media: nutrient agar, 5 % human blood agar, MacConkey agar, and chocolate blood agar .colonies were subcultured to tryptone soy agar slants and the organisms were identified to the level of species by using conventional biochemical tests (12) then the identification was confirmed using API systems strips as recommended by Biomérieux (France).

The resistance of the bacterial isolates to antimicrobial agents was determined by using disk diffusion method (13) and interpreted according to clinical and laboratory standards institute – CLSI-documents (14).

The following antimicrobial agents were obtained (from Oxoid, U.K.) as standard reference disks as known potency for laboratory use: Penicillin (P) 10 units, Ampicillin (Amp)10 μg, Amoxicillin (Amx) 10 μg, Cephalexin (K) 30 μg, Cefotaxime (Ctx) 30 μg, Ceftizoxime (Czx)30 μg, Gentamycin (Gm) 10 μg, Streptomycin (S) 10 μg, Erythromycin (E)15 μg, Chloramphenicol (C)30 μg, Tetracycline (Te) 30 μg, Lincomycin (L), Rifampicin (R) 5 μg , Polymyxin B (PB) 300 units, Bacitracin (B), 10 μg, and Trimethoprim–Sulfamethoxazole (SXT) 1.25-23.75 μg.

All these tests were performed on plates of Muller- Hinton agar (Oxoid, U.K.).
A 0.5 McFarland suspension (provided by Biomérieux/ France) of tested bacterial isolates was applied to the plates, which were dried in an incubator at 35 ºC for 15 minutes. Antimicrobial disks were placed on the agar with sterile forceps.The agar plates were incubated inverted at 35 ºC for 18 hours. Results were

recorded by measuring the inhibition zone (in millimeters) and interpreted according to Clinical and Laboratory Standards Institute documents (14).

RESULTS:

Isolation and identification of bacterial isolates:

Gram- positive rods were the most commonly isolated bacteria from oral and topical medicaments (12.7 %) followed by Gram- positive cocci (11.7 %). Gram- negative rods were isolated only from topical medicaments. Topical medicaments showed high proportion of contaminated samples (17.6 %) and more bacterial genera than oral ones (Table- 1).

Fifty three bacterial isolates were isolated that belonging to the following genera; *Bacillus* (29 isolates), Staphylococcus (11 isolates), *Micrococcus* (5 isolates), *Escherichia* (3 isolates), *Klebsiella* (2 isolates), *Enterobacter* (2 isolates), and *Pseudomonas* (1 isolate). Three species of *Bacillus* were identified, the most frequent species was *B. licheniformis* (Table- 2).

The most frequent species of *Staphylococcus* isolated from oral and topical medicaments was *S. epidermidis*. One isolate of *S. aureus* was detected in one sample of ointments (Table-3).

Among Gram- negative rods isolated from topical pharmaceuticals, three isolates of faecal indicator (*Escherichia coli*) were found in one sample of topical medicaments. One isolate of *Pseudomonas aeruginosa* was also detected in one sample of topical medicaments (Table- 4).

Resistance to the antimicrobial agents:

The antibiotic resistance patterns of the bacterial isolates recovered from oral and topical medicaments was determined (Tables 2,3,and 4).

Bacillus isolates were resistant to lincomycin (58.6 %) and Bacitracin (20.6 %) but they were all susceptible to Gentamycin and Rifampicin . *B. mycoides* showed the highest number of isolates resistant to Penicillins, Cephalosporins, and Trimethoprim- sulfamethoxazole (STX), while *Bacillus* spp. isolates were susceptible to all these antimicrobial agents (Table-2).

A high percentage of *Staphylococcus* isolates were highly resistant to Amp (72.7 %), Amx. (63.6 %), P. (54.5%), and STX (54.5 %) but they were all susceptible to Extended-spectrum beta-lactams (Cefotaxime and Ceftizoxime), Aminoglycosides (Gentamycin and Streptomycin), and Lincomycin.

S. epidermidis has the highest number of multi-resistant strains. *S. aureus* strain was multi-resistant to Beta-lactams, Tetracyline, and SXT (Table- 3). *Micrococcus* strains were susceptible to all antimicrobial agents used in this study except for Strepyomycin and Chloramphenicol.

A high percentage of Gram-negative rods strains were highly resistant to Beta-lactams and Erythromycin. *P. aeruginosa* isolate showed multi-resistance to Beta-lactams, Gentamycin, and

Erythromycin antibiotics (Table-4). *E. aerogenes* isolates showed multi-resistance to Beta-lactams, tetracycline, and Streptomycin. *Klebsiella pneumoniae* isolate showed, in addition to Betat-lactams and Erythromycin, resistance to extended-spectrum beta-lactams (Table- 4).

Discussion:

The results showed that *Bacillus* species were the bacteria most frequently found in orally and topically administrated medicaments, and this in agreement with the work of other investigators (1 , 2).

The significant microbial contamination of non-sterile pharmaceuticals with these bacteria is attributed to that *Bacillus* species are widely distributed in the soil, dust, air, and water and because they are resistant to environmental destructive factors (15). Although the members of the genus *Bacillus* have been frequently considered as non-pathogenic, some authors have reported serious human infections associated with *Bacillus* spp. particularly in immunosuppressed patients (16).

Like *Bacillus* spp., Gram- positive cocci can survive in the environment and thus contaminate the medicaments. *S. epidermidis* was the most frequently isolated species from oral and topical medicaments (1, 3). *Micrococcus* spp. were also isolated from liquid and solid drugs (1).

The presence of Gram- negative rods in the non-sterile medicaments was expected because of their resistance to the antimicrobial agents and their high metabolic activity.

The isolation of *P. aeruginosa* from topical medicament was similar to the findings presented by Spooner (5). The contamination of the medicaments with these bacteria may pose an important and serious problem because it may generate a severe infections to human. de la Rosa and her colleagues (1) found several *Pseudomonas* strains, but they did not isolate *P. aeruginosa* from pharmaceuticals they studied them.

Other Gram- negative rods were also isolated from non-sterile topical medicaments, these have been *E. coli, Klebsiella* spp., and *E. aerogenes*. Similar finding were also reported by other authors (1, 5, 6).

Results of this study about resistance of *Bacillus* isolates to antimicrobial agents were similar to those medicament (1) and clinical isolates (17).

The high proportion of resistant isolates to lincomycin refers that this antibiotic is not the most suitable antibiotic for these bacteria and this conclusion is similar to the opinion of de la Rosa et. al. (1).

Staphylococcus isolates were resistant to penicillins and this may be due to their ability to produce penicillinase enzyme or by changing their PBPs. *S. epidermidis* had the highest number of mutiresistant isolates and these findings are in agreement with those isolated from clinical specimens (18).

S. epidermidis may acts as a reservoir for resistance which can be transferred to *S. aureus*. The transfer of resistance among different genera of Gram-positive and Gram-negative bacteria and between *Bacillus* species and Staphylococci has been reported by many authors (19).

Results showed that the strain of *S. aureus* isolated in this study was multi-resistant to Beta- lactams ,Te, and SXT, and this in agreement with several studies of clinical strains (9) but disagree with studies of medicament strains reported by other authors (1).

The high level of resistance to many antimicrobial agents shown by Gram- negative rods is well known and has been reported with increasing frequency (20). *Klebsiella*, *E. coli*, and *Enterobacter* are of increasing importance, as resistance to newer beta-lactams particularly extended-spectrum beta-lactams may be acquired by mutation in addition to plasmids (21, 22).

Resistance to beta-lactam antibiotics in Gram- negative bacteria can be due to four mechanisms: decreased permeability of the drug into the cell, hydrolysis of the drug by ß-lactamase, decreased affinity of the target penicillin-binding proteins (PBPs), and efflux pumps mechanism (22).

The major mechanism of resistance in bacteria causing clinically significant infection remains the expression of ß-lactamases, of which there are several classes including plasmid- encoded and chromosomally- encoded enzymes (22).

E. coli and *E. aerogenes* isolates showed multi-resistance to beta-lactams and Erythromycin. These findings are similar to those of clinical strains reported by several investigators (20). *Klebsiella* spp. showed, in addition to these antibiotics, resistance to extended-spectrum beta-lactams (Ctx and Czx). These results are in agreement with those of clinical strains reported by several authors (20). The only one isolate of *P. aeruginosa* from topical medicament was multi-resistant to beta-lactams, Gentamycin and Erythromycin. This result is similar to clinical strains reported by several authors (20).

The results of this study indicate a high rate of resistance to antimicrobial agents of bacterial isolates from oral and topical medicaments, and this may indicate a widespread antibiotic resistance among bacteria isolated from different sources, including that of anthropological and environmental origin.

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Table 1. Number and percentage of contaminated samples of oral and topical medicaments.

Bacterial isolates	**Medicaments (No. of samples)**					
	Oral (85)		**Topical (17)**		**Total (102)**	
	Number	**%**	**Number**	**%**	**Number**	**%**
Gram+ ve rods:						
Bacillus	10	11.7	3	17.6	13	12.7
Gram+ ve cocci:						
Staphylococcus	7	8.2	2	11.7	9	8.8
Micrococcus	2	2.3	1	5.8	3	2.9
Gram-ve rods:						
Pseudomonas			1	5.8	1	0.9
Escherichia coli			1	5.8	1	0.9
Klebsiella			1	5.8	1	0.9
Enterobacter			1	5.8	1	0.9

Table 2. Antibiotic resistance of *Bacillus* isolates recovered from medicaments

Species	**No. of isolates**														
	Oral	**Topical**	**Total**	**P**	**Amp**	**Amx**	**K**	**Ctx**	**Czx**	**C**	**Te**	**SXT**	**L**	**PB**	**B**
B. licheniformis	2	4	6	1	1	1				1			3	1	3
B. mycoides	2	3	5	4	3	3	2	1	2		2	3	2		
Bacillus spp.	12	6	18							3			12		3
Total No.	18	11	29	5	4	4	2	1	2	4	2	3	17	1	6
Total %				17.2	13.7	13.7	6.8	3.4	6.8	13.7	6.8	10.3	58.6	3.4	20.6

Table –3. Antibiotic resistance of *Staphylococcus* and *Micrococcus* isolates isolated from medicaments

Species	**No. of isolates**											
	Oral	**Topical**	**Total**	**P**	**Amp**	**Amx**	**K**	**S**	**E**	**C**	**Te**	**SXT**
Staphylococcus epidermidis	4	3	7	3	5	4			1	2	4	3
Staphylococcus aureus		1	1	1	1	1	1				1	1
Staphylococcus spp.	2	1	3	2	2	2					1	2
Micrococcus spp.	2	3	5					2		2		
Total No.	8	8	16	6	8	7	1	2	1	4	6	6
Total %				37.5	50	43.7	6.2	12.5	6.2	25	37.5	37.5

Table 4. Antibiotic resistance of Gram-negative rods isolates recovered from topical medicaments

Species	No. of isolates	No. of resistant isolates									
		P	**Amp**	**Amx**	**K**	**Ctx**	**Czx**	**Gm**	**S**	**Te**	**SXT**
Pseudomonas aeruginosa	1	1			1			1			
Escherichia coli	3	2	3	2	2			1	1		
Klebsiella pneumoniae	2	2			2	1	1				1
Enterobacter aerogenes	2	2							1	2	
Total No.	8	7	7	7	5	1	1	2	2	2	1
Total %		87.5	87.5	87.5	62.5	12.5	12.5	25	25	25	12.5