

Incidence and Antibiotics Sensitivity of Multidrug-Resistance of *Pseudomonas aeruginosa* Isolated from Burn's Patients and Environmental Samples from Three Hospitals in Baghdad

المقاومة المتعددة للمضادات الحيوية لإصابة الزائفة الزنجارية المعزولة من مرضى الحروق والعينات البيئية لثلاث مستشفيات في بغداد

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Abstract

Two hundred swab samples were collected from burn patients and burn units for the period, beginning from August 2012 to the end of April 2013 from a number of hospitals in Baghdad governorate including: Al-Kindi General Teaching Hospital, Al-Yarmuk General Teaching Hospital and Al-Imam Ali Hospital. The collected samples were cultured on different media and tested biochemically in order to find out the profile of bacteria that colonize burn patients wounds and environment of burn units. The results of bacterial culturing revealed that out of 200 samples, 105 samples 52.5% were observed to have bacterial growth (positive samples), while negative samples represented 95(47.5%). The profile of the bacteria in the cultured samples revealed: *Pseudomonas aeruginosa* 40.95% was the most common isolate followed by *Staphylococcus aureus* 20.0%, *Klebsiella pneumoniae* 17.1%, *Escherichia coli* 8.5%, *Pseudomonas putida* 4.76%, *Enterobacter aerogenes* 3.80%, *Acinetobacter baumannii* 2.85% and *Proteus mirabilis* 1.90%. Forty three *P. aeruginosa* isolates were tested for antibiotic susceptibility. The results showed most isolates were potentially resistant to different antibiotics as follow, all isolates 100% had resistance to Ceftriaxone, Cefepime, and Chloramphenicol, and showed high resistance to Tobramycin 95.3%, Gentamicin 93.0%, Ceftazidime 88.3%, Cefotaxime 86.0%, Piperacillin 83.7% and Amikacin 79.0%, beside illustrating low resistance to Aztreonam 67.4%, Ciprofloxacin 46.5%, and Imipenem 13.9% among these antibiotics, Imipenem was the most effective antibiotic because 86.0% of the isolates appeared to be high sensitive to it.

Key words: Antibiotics Sensitivity of Multidrug-Resistance, *Pseudomonas aeruginosa*, Burn's Patients

المخلص

جمعت مائتي مسحة من مرضى الحروق ووحدات الحروق للفترة من بداية آب 2012 إلى - نهاية نيسان 2013 من عدد من المستشفيات في محافظة بغداد منها: مستشفى الكندي التعليمي العام ومستشفى اليرموك التعليمي العام و مستشفى الإمام علي. زرعت العينات على الأوساط الزرعية المختلفة وخضعت للاختبارات الكيموحيوية للتعرف على البكتيريا التي تستعمر مرضى الحروق وبيئة وحدات الحروق. كشفت نتائج الزراعة البكتيرية ان هناك نمو بكتيري لـ 105 (52.5%) عينة من مجموع 200، بينما مثلت 95 (47.5%) عينة نتيجة سالبة للنمو. عينات العزلات الأكثر شيوعاً تمثلت بكتيريا *Pseudomonas aeruginosa* (40.95%) يليها *Staphylococcus aureus* (20.0%)، *Klebsiella pneumoniae* (17.1%)، *Escherichia coli* (8.5%)، *Pseudomonas putida* (4.76%)، *Enterobacter aerogenes* (3.80%)، *Acinetobacter baumannii* (2.85%) وأخيراً *Proteus mirabilis* (1.90%). تم اختبار حساسية جميع عزلات *P. aeruginosa* (43) لاثنتي عشر نوعاً من المضادات الحيوية، وكانت معظم العزلات مقاومة لمختلف هذه المضادات وكما يلي، جميع العزلات (100%) مقاومة السيفترياكسون، الكلورامفينيكول والسيفيبيم، وأظهرت مقاومة عالية تجاه، توبراميسين (95.3%)، جنتاميسين (93.0%)، سيفتازيديم (88.3%)، سيفوتاكسيم (86.0%)، بيبيراسيلين (83.7%) وأخيراً الأميكاسين (79.0%). في حين كانت المقاومة أقل ضد الأتريونام (67.4%)، سيبروفلوكساسين (46.51%)، والإميبينيم (13.9%). كان المضاد الحيوي الإميبينيم الأكثر فعالية ضد عزلات *Pseudomonas aeruginosa* حيث أبدت (86.0%) من العزلات حساسية عالية لهذا المضاد الحيوي.

الكلمات المفتاحية: المقاومة المتعددة للمضادات الحيوية، الزائفة الزنجارية، مرضى الحروق.

Introduction

Infections are the major cause of morbidity and mortality in burn patients. Three fourth of deaths in burn patients occur due to infections. Skin is one of the largest organs in the body, performs numerous vital functions, including fluid homeostasis, thermoregulation, immunologic functions, neuro sensory functions, and metabolic functions (e.g., vitamin D) [1]. Skin also provides primary protection against infection by acting as a physical barrier, when this barrier is damaged; pathogens have a direct route to infiltrate the body, possibly resulting in

infection [2]. Burns provide a suitable site for bacterial multiplication. Moreover the larger area of tissue is exposed for a longer time that renders patients prone to invasive bacterial sepsis [1]. The pathogens that infect the wound are primarily gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and gram-negative bacteria such as complex, *Pseudomonas*, and *Klebsiella* species. These latter pathogens are notable for their increasing resistance to a broad array of different antimicrobial agents [3]. The risk of infections in burns is well known, available current techniques of burn wound care have significantly decreased the incidence of infections in patients with burn wounds [4]. Gram-negative pathogens continue to cause the most severe infections in burn's patients. Among these organisms, *P. aeruginosa* is the most commonly encountered source of chronic or acute burn wound infection in the United States [5]. In Europe, *P. aeruginosa* and *E. coli* are the two most common pathogens, with a frequency for each at 13% of all gram-negative infections [6]. In recent years, emerging resistant pathogens have forced burn care providers worldwide to search for alternative forms of treatment. Multi-drug resistant *S. aureus*, *P. aeruginosa*, *Acinetobacter spp.*, and various fungal strains have been the major contributors to the increase in morbidity and mortality rates [5, 6]. Microorganisms routinely isolated from burn injuries and wounds include aerobic organisms like *P. aeruginosa*, *S.aureus*, *E.coli* ...etc. according to data from various medical records in different countries, the epidemiology of the pathogens of burn wounds is represented by: *P.aeruginosa* (25. 74%), *S.aureus* (9.17%), *E. coli* (5.35%) ..etc [7]. In Iraq, Alwan [8] studied the bacteria of burns wounds and their antimicrobial susceptibility; it was found *P. aeruginosa* the most common isolate, followed by *S. aureus*. *Pseudomonas aeruginosa* was the most commonly gram-negative bacilli isolated from burns, followed by *E. coli*, *Enterobacter spp.*, *Klebsiella spp.* and *Proteus spp.* This study was conducted to determine the incidence of *Pseudomonas aeruginosa* infections and sensitivity of antibiotic among burn patients for nine months. Burns are injuries to tissues caused by heat, electricity, radiation or other factors [9].

The Aim of Study

This study was conducted to determine the incidence of *P. aeruginosa* infections and sensitivity of antibiotic among burn patients.

Materials and Methods

Collection of Samples

This study last for nine months starting from August 2012, till April 2013. The clinical swabs were collected from 100 burn's patients in three hospitals in Baghdad, Al-Kindi General Teaching Hospital 59 swabs, Al-Yarmuk General Teaching Hospital 22 swabs and Al-Imam Ali Hospital 19 swabs. Population of study included both gender with different age started with younger patients who were one year old and ended with oldest patients who were seventy one years old, admitted patients came from different geographic residencies, and one hundred environmental swabs were taken from equipments of burn's units: gloves, beds, floors, benches, walls and washing baths from different hospitals as following: 40 swabs from Al-Kindi General Teaching Hospital, 40 swabs from Al-Yarmuk General Teaching Hospital and 20 swabs from Al-Imam Ali Hospital.

Isolation of Bacteria

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas putida*, *Enterobacter aerogenes*, *Acinetobacter baumannii* and *Proteus mirabilis* were isolated from 200 samples which isolated from burn's patients skin and burn's units equipments of the three hospitals in Baghdad/Iraq. All collected swabs were cultured on MacConkey agar and Blood agar, incubated aerobically at 37°C for 24 hr and citrimide agar at 42°C [10]. Only the cultured swabs which gave growth on these media were estimated to have positive results.

Identification of Bacterial Isolates

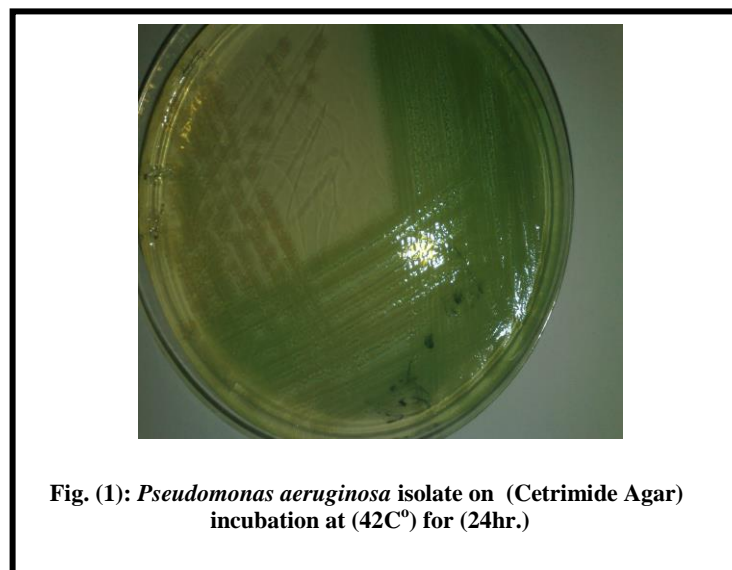
Staphylococcus aureus grew on mannitol salt agar which was a selective medium, isolated and identified biochemically, and gave positive results for catalase and coagulase tests and by API Staph. All gram negative bacteria identified by traditional biochemical tests and API 20 E including *K. pneumonia*, *E. coli*, *P. putida*, *E. aerogenes* and *A. baumannii*,. *Pseudomonas aeruginosa* isolates were identified by traditional biochemical tests including Oxidase, Catalase, IMVC, Citrate utilization, Urease Production, Motility, Growth on Citrimide Agar and growth at 42°C. In addition to these tests, Sugar fermentation tests including Glucose, Sucrose, Maltose, and by API 20 E standardized identification system were also performed [10]. In this study we used 12 antibiotics susceptibility testing by a standardized single disk method as previously published by [11]. Gram stain bacteria was significantly higher than positive in both burn's patients and burn's units.

Results and Discussion

The results of bacterial culture obtained from 100 burn's patients admitted by the three hospitals clarified that, 67 (67%) gave positive result while 33 (33%) gave negative result. Thirty three percentages 33% of cases showed negative culturing that may be due to either patients have antibiotics treatment swabs were obtained from new burns because burn surfaces were initially sterile, but within 48hr the wound was typically colonized by microorganisms [12]. The present study revealed that 40(59.7%) from total positive culture showed single bacterial isolate while the results clarified that, *P. aeruginosa* was the commonest isolate (28 isolates; 41.8%) followed by *S. aureus* (13 isolates; 19.4%), *K. pneumonia* (12 isolates; 18%), *E. coli* (6 isolates; 8.9%), *P. putida* (3 isolates; 4.5%). Both *E. aerogenes* and *A. baumannii* were 2 isolates for each (3.0%), and *P. mirabilis* the lowest isolated microorganisms which only account for one (1.5%), Table (1) and Figure (1) represent *P. aeruginosa* isolate cultured on Cetrimide Agar.

Table (1): Types of Clinical Isolates From Burn's Patients Wounds.

Pathogens isolation	Total Viable Count	Percentage (%)
<i>Pseudomonas aeruginosa</i>	28	41.8
<i>Staphylococcus aureus</i>	13	19.4
<i>Klebsiella pneumonia</i>	12	18.0
<i>Escherichia coli</i>	6	8.9
<i>Pseudomonas putida</i>	3	4.5
<i>Enterobacter aerogenes</i>	2	3.0
<i>Acinetobacter aumannii</i>	2	3.0
<i>Proteus mirabilis</i>	1	1.5
Total No.	67	100.1



API 20 E system used for confirmation the diagnosis of *P. aeruginosa* isolates. Figure (2) Showed biochemical reactions of these bacteria after 24 hr at 37°C.



The cultural results of 100 environmental swabs collected from burn's units equipments (gloves, beds, floors, benches, walls and washing baths) from the three hospitals, revealed that 38 (38%) of swabs gave positive result for bacterial growth and the rest 62 (62%) were negative. The predominant bacteria was *P. aeruginosa* (39.50%),

followed by *S. aureus* (21.0%) while *K. pneumoniae* came third (15.7%), then *E. coli* (7.8%), *P. putida*, *E. aerogenes* recovered in similar rate (5.26%), the least isolated microorganism were *A. baumannii* and *P. mirabilis* as (2.63%) for each, Table (2).

Table (2): Types of Environmental Isolates from Burn's Units Equipments.

Isolate	Total Viable Count	Percentage (%)
<i>Pseudomonas aeruginosa</i>	15	39.5
<i>Staphylococcus aureus</i>	8	21.0
<i>Klebsiella pneumonia</i>	6	15.7
<i>Escherichia coli</i>	3	7.8
<i>Pseudomonas putida</i>	2	5.26
<i>Enterobacter aerogenes</i>	2	5.26
<i>Acinetobacter baumannii</i>	1	2.63
<i>Proteus mirabilis</i>	1	2.63
Total No.	38	100

The results of bacterial culturing revealed that out of 200 samples isolates from burn's patients and burn's units equipments, 105 samples (52.5%) were observed to have bacterial growth (positive samples), while negative samples represented (47.5%). Gram positive bacteria were observed in 19.4% of clinical isolates, while the corresponding percentage of environmental isolates was 21.0%. The remaining samples were represented G-ve bacteria and their percentage in Clinical isolates and environmental isolates were 80.6% and 79.0%, respectively. The number of negative Gram stain bacteria was significantly higher than positive in both burn's patients and burn's units ($P \leq 0.001$) as illustrated in Table (3).

Table (3): Distribution of (G+ve) and (G-ve) Bacteria of Clinical and Environmental Isolates.

Samples	Total Viable Count				Total No. (%)		X ²	P
	G+ve		G-ve		No.	%		
	No.	%	No.	%				
Clinical isolates	13	19.4	54	80.6	67	100.0	17.655	< 0.001
Environmental isolates	8	21.0	30	79.0	38	100.0	13.564	< 0.001

Cultural evaluation of samples with a growth of aerobic bacteria revealed that there were eight main pathogens (*P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *E. coli*, *P. putida*, *E. aerogenes*, *A. baumannii*, and *P. mirabilis*), which were isolated and identified. Such distribution was different with the source of sample (burn's patients and burn's units) was considered. It was found that the *P. aeruginosa* consist 41.8% of clinical isolate (burn's patients) and 39.5% of environmental isolates (burn's units), followed by *S. aureus* 19.4% of clinical isolates and 21% of environmental isolates and so on as demonstrated in Table (4).

Table (4): Identification Percentages of Bacterial Isolates from Cultured Burn's Patients and Environment Burn's Units Samples.

Pure Isolated Bacteria	Total Viable count Isolated from Burn's Patients and Environment Burn's Units	
	No.	%
<i>Pseudomonas aeruginosa</i>	43	40.95
<i>Staphylococcus aureus</i>	21	20.0
<i>Klebsiella pneumonia</i>	18	17.1
<i>Escherichia coli</i>	9	8.5
<i>Pseudomonas putida</i>	5	4.76
<i>Enterobacter aerogenes</i>	4	3.80
<i>Acinetobacter baumannii</i>	3	2.85
<i>Proteus mirabilis</i>	2	1.90
Total	105	100

In this study, *P. aeruginosa* was found to be the most incidence bacterium in burn's patients and burn's units, so this result was expected since this bacterium is resistant to many antibiotics and antiseptics. This bacteria occurs so commonly in the environment making it extremely likely that an individual suffering severe burns or contaminated of environment burn's units will be challenged with this opportunistic microorganism before the burn can heal [13]. *Pseudomonas aeruginosa* has been found to contaminate the floors, bed rails and sinks of hospitals, and has been also cultured from the hands of nurses [14]. Besides transmission through vomits and vectors, bacterial flora can be carried into a hospital by the patients and can be an important source of infection

for the same individual after incineration [15]. These potential pathogens typically come from the patients gastrointestinal tract, upper respiratory tract, or the hospital environment, transferring through contact with health care workers. Fungal infections often develop later [2]. *Pseudomonas aeruginosa* survives well in the hospital environment, once it was established, it can persist for months within a unit, posing as Multi drug resistant nosocomial infection risk for patients being treated [16]. Hands of staff members can become transiently contaminated and transfer infection among patients that play important role in increase chance of spreading this microorganisms [17], also this high frequency of *P. aeruginosa* might be due to prolonged hospital stay and intensive use of antibiotics [18]. *Staphylococcus aureus* emerged as a second pathogen was isolated with 20.0% of total isolates this does not coincidence with the findings of Al-Khazali who found gram positive bacterium *S. aureus* (57.6%) was the most incidence in burn's patients and burn's units [19]. *Klebsiella pneumoniae* was obtained from burn's patients and environment burn's units with percentage of 17.1%, this result coincidence with Al-Shamary who found it was (22.4%) the second most common isolate in burn's injuries and units [20]. *Proteus mirabilis* found in percentage of 1.90% and that similar to results which reported *P. mirabilis* with 2.1% [20]. The variations among studies in the type and the rate of bacterial isolation from burn's patients and environment burn's units may be due to the varies of infective agents spectrum from time to time and from place to place. Also these variations effected by patient hospitalization period. Also antimicrobial susceptibility was performed to 43 *P.aeruginosa* isolates of 12 antibiotics, 7 of them were extended spectrum beta lactamase (ESBLs) represented by Cefotaxime, Ceftriaxone, Ceftazidime, Imipenem, Aztreonam, Piperacillin and Cefepime, and to 5 antibiotics were non ESBLs represented by Aminoglycoside (Amikacin, Gentamicin and Tobramycin), Chloramphenicol and Fluoroquinolone (Ciprofloxacin), by the disc diffusion method (DDM), as described by [11]. The antibiogram for studied isolates was revealed that all isolates (100%) resist to Ceftriaxone, Cefepime, and Chloramphenicol and this resistance became 95.3 and 95.3% against Tobramycin and Gentamicin, while reached to 93.0 , 88.3 and 86.0% against Ceftazidime, Cefotaxime and Piperacillin respectively, followed by 81.3% for Amikacin and lower resistance 67.4% and 48.8% for Aztreonam and Ciprofloxacin respectively, Imipenem was the most effective antibiotic 86.0% of isolates appeared to be high sensitive to it. *Pseudomonas aeruginosa* was becoming resistant to commonly used antibiotics and gaining more and more resistance to newer antibiotics [12]. This study found that Imipenem is the drug of chose in treatment of *P. aeruginosa* burn injury, because 86.0% of isolates were susceptible to it and only six isolate were exhibit resistance, as illustrated in Table (5) and Figure (3) represent susceptibility paterren of *P. eruginosa* against various antibiotics.

Table (5): Antibiotic Susceptibility of *Pseudomonas aeruginosa* Isolates.

Antibiotics	Disk Content	<i>P. aeruginosa</i> isolates of total number viable count = 43 q2					
		Resistant		Zone diameter (mm)	Sensitive		Zone diameter (mm)
		No. of Bacteria	%		No. of Bacteria	%	
Cefotaxime	30µg	38	88.3	≤14	5	11.6	≥23
Ceftriaxone	30µg	43	100.0	≤13	-	-	≥21
Ceftazidime	30µg	40	93.0	≤14	3	6.9	≥18
Imipenem	10µg	6	13.9	≤13	37	86.0	≥16
Aztreonam	30µg	29	67.4	≤15	14	32.5	≥22
Amikacin	30µg	35	81.3	≤14	8	18.6	≥17
Gentamicin	10µg	41	95.3	≤12	2	4.6	≥15
Ciprofloxacin	5µg	21	48.8	≤15	22	51.1	≥21
Piperacillin	100µg	37	86.0	≤17	6	13.9	≥18
Cefepime	30µg	43	100	≤14	-	-	≥18
Chloramphenicol	30µg	43	100	≤12	-	-	≥18
Tobramycin	10µg	41	95.3	≤12	2	4.6	≥21

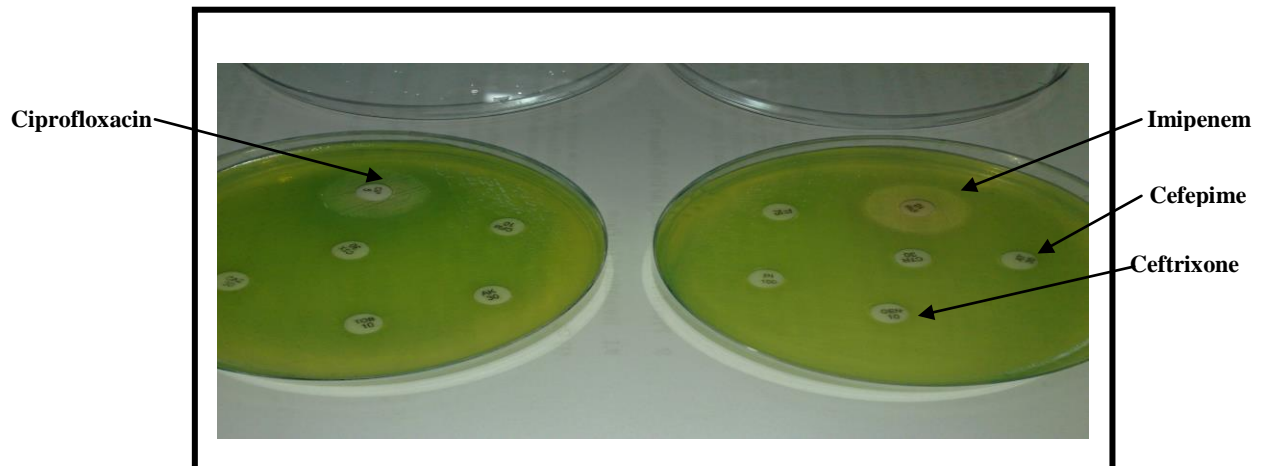


Fig. (3): Susceptibility Pattern of *P. aeruginosa* against various antibiotics according to (Disc-Diffusion Method) culture on (Cetrimide Agar) incubation at (42C°) for (24hr.)

The results have shown sensitivity of *P.aeruginosa* against Imipenem reached to 100% , 86.1% and 66.7% respectively these results were similar to several studies [21]. Ciprofloxacin has been reported as the second most effective drug against *P. aeruginosa* with sensitivity reached to 51.1% and agreed with a study done in Chandigarh-India [22]. Aztreonam was a monobactam β -lactam drug, It has excellent activity against *Pseudomonas* species but has a limited treatment option against Multidrug-resistant (MDR) strains of *P. aeruginosa* [23]. Aztreonam was used in the present study and it's sensitivity reached to 32.5%. Piperacillin was active only against 13.9% of isolates. This finding was unique from other studies in which 57.4% of *P.aeruginosa* isolates susceptible to Piperacillin [24]. This study investigated that four ESBLs antibiotics the least effective against *P.aeruginosa* and the resistance reached to 86.0% and 88.3% for Cefotaxime and Ceftazidime while was 100% for both Ceftriaxone and Cefepime 100%. In the present study 93.0% of *P. aeruginosa* isolates showed resistance against antibiotics other than ESBLs such as, Gentamicin, this finding is similar to Iranian study that shown more than 95% strains of *P. aeruginosa* were resistant to Gentamicin , Gentamicin is a cheap and easily available drug that is used extensively in general and hospital practice in clinically suspected Gram negative infections. This may be the main reason for the development of resistance in bacteria against this drug. *P. aeruginosa* has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance, and several epidemiological studies confirmed its occurrence as a nosocomial pathogen and indicated that antibiotic resistance was increasing in clinical isolates toward most antibiotics [25].

Recommendations

Based on the findings of the present study, after reviewing the literature and many of the previous trials, the following recommendations can be suggested: Studying the changes in the pattern of bacterial colonized burns patients and environment burn units during the period of hospitalization. Using disinfectants in the hospitals to get rid of contamination and reduce the spread of antibiotic resistance.

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