



Antibiotic Resistance Profile of Bacterial Isolates Cultured from Urine Samples of HIV Seropositive Pregnant Women

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OVO, ABA and ADO were responsible for study design and supervision of work. Author OVO was responsible for laboratory work. Authors OVO, ABA and ADO were responsible for data analysis and manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the antibiotic resistance pattern of some bacterial isolates cultured from urine samples of HIV seropositive pregnant women that attended antenatal clinic of the Ondo State Specialist hospital, Akure. The study determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of some antibiotics used against these bacterial isolates. The results showed the MIC and MBC values obtained for augmentin were found to be highest in *Staphylococcus aureus* (22.8 mg/mL) and lowest against *Escherichia coli* (1.43/2.85 mg/mL). The MIC and MBC values of all the antibiotics used against *S. aureus* isolates ranged from 0.004/0.008 mg/mL to 22.8 mg/mL. The MIC and MBC values of all the antibiotics tested against *E. coli* isolates ranged from 0.008/0.016 to 11.4/12.5 mg/mL, while the MIC and MBC values of all the antibiotics tested against *Pseudomonas* spp ranged from 0.391/0.781 mg/mL to 11.4/12.5 mg/mL. Among all the antibiotics used, ciprofloxacin was more effective against the bacterial isolates tested. The study concluded that most of the bacterial isolates cultured in this study were multi-resistant to different antibiotics tested *in vitro*.

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1. INTRODUCTION

Antimicrobial resistance (AMR) is a global public health crisis with obvious association between infection and increased morbidity and mortality [1]. Resistance is referred to when an organism previously or originally sensitive to an antibiotic suddenly becomes resistant to the same dose and type of the antibiotic [2]. Resistant organisms (bacteria, fungi, viruses and some parasites) are able to withstand attack by antimicrobial agents, such as antibiotics, antifungals, antivirals, and antimalarial, so that standard treatments become ineffective and infections persistently increasing the risk of spreading to others [2]. It is generally considered to be a consequence of the wide use and misuse of antibiotics [1].

However, there has been a continual battle between humans and the multitude of microorganisms that cause infection and disease like Bubonic plague, tuberculosis, malaria, and more recently, the human immune deficiency (HIV) virus or acquired immunodeficiency syndrome (AIDS) pandemic have affected substantial portions of the human population, causing significant morbidity and mortality [3]. Antibiotic resistance occurs when bacteria change in some ways that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection thus, the bacteria survive and continue to multiply causing more harm [4]. Compounding the problem of rising bacterial resistance to currently approved antibiotics is a lack of investment in antibiotic discovery by the pharmaceutical industry due to the inherently low rate of return for antibiotics compared to drugs targeted at chronic diseases [5]. This situation is so dire that the World Health Organization has identified multidrug resistant bacteria as one of the top three threats to human health and the Infectious Disease Society of America has issued a call to action from the biomedical community to deal with the multidrug resistant bacteria threat [6,7].

The development of new antibiotics is one approach for the treatment of multidrug resistant bacterial infections, the fact remains that only two new classes of antibiotics has been introduced in to the clinic over the past two decades; neither of which are significantly active against Gram-negative bacteria [8]. Furthermore, bacteria

invariably develop resistance to any introduced therapy that relies solely upon a bacteriostatic or bactericidal mechanism and clinically significant resistance can appear in a period of just months to years following introduction of a new antibiotic into the clinic [9,10].

In developing countries like Nigeria, the antibiotic resistance may cause public health phenomenon in association with infections which can lead to morbidity and mortality in immune compromised patients most especially HIV seropositive pregnant women and since the pregnancy is also a predisposing factor to a compromised immune status. More so, there is limited information on the antibiotic resistance profile of significant bacteria associated with HIV seropositive pregnant women hence, this study intends to characterize and determine the antibiotic resistance profile of bacterial isolates cultured from the urine of such subjects.

The aim of this study was to evaluate the antibiotic resistance of urine bacterial isolates cultured from HIV seropositive pregnant women and to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of some antibiotics used against these isolates.

2. METHODOLOGY

2.1 Materials

Bacterial isolates (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonasa eruginosa* and *Pseudomonas fluorescens*) cultured from the urine samples of HIV seropositive pregnant women attending antennal clinic at the Ondo State Specialist Hospital, Akure, South western, Nigeria were used for the study. Each bacterial isolate was verified using cultural morphology, Gram's staining, selective media and differential media as well as biochemical tests to authenticate each isolate's identity (Monica Cheese Brough).

2.2 Preparation of Antibiotic Stock Solutions

The following classes of antibiotic (Beecham and GSK powder form) penicillin, cephalosporin, fluoroquinolone, macrolide (erythromycin), aminoglycoside (gentamicin), tetracycline and chloramphenicol were used. Each antibiotic was

dissolved separately in sterile solvent/diluent (water was used as solvent for the majority of the antibiotics) except the erythromycin that dissolved in DMSO according to methods of Jennifer [11] and the concentration of each antibiotic employed was based on the manufacturer's specifications for preparing antibiotic stock solutions in mg/ml.

2.3 Determination of MIC and MBC

Determination of Minimum Inhibitory Concentration (MIC) of the representative of each class of antibiotic was carried out using the tube dilution method (10^1 to 10^8 serial dilutions) [11,12]. This involved ten test tubes containing 1 ml of sterile Mueller Hinton broth each which were placed on the rack. The test tube one on the rack as antibiotic control (A.C) and growth control (G.C) was test tube 10 respectively. Test tube number 1 to 8 which served as experimental. A serial dilution of each antibiotic in sterile Mueller Hinton broth was carried out. Each test tube was inoculated including the growth control (except antibiotic control) with 1 ml of each bacteria isolate and was incubated at 37°C for 24 hours and the MIC was thereafter monitored. The Minimum Bactericidal

Concentration (MBC) was done by subculture all the test tubes with which growth was absent on nutrient agar that not contained any antibiotics which was incubated at 37°C for 24 hours and the MBC was thereafter monitored.

3. RESULTS

3.1 Identification of the Bacterial Isolates

The bacterial isolates that were screened include *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* spp. Altogether, a total number of fifty (50) isolates were screened with *S. aureus* accounting for twenty 20 (40%), *E. coli* accounting for ten 10 (20%) and *Pseudomonasa eruginosa* and *Pseudomonas fluorescens* being 20 (40%).

3.2 Determination of MIC and MBC

The results showed that almost all the bacterial isolates recovered from urine samples of HIV seropositive pregnant women gave varying MIC values. Some of the bacterial isolates were inhibited killed at the same concentrations, while majority of these isolates were killed at relatively high concentrations.

Table 1. Profile of MIC and MBC of penicillin for *Staphylococcus aureus* isolates

S/N	Isolates code	MIC for penicillin (mg/mL)		MBC for penicillin (mg/mL)	
		Augmentin	Amoxicillin	Augmentin	Amoxicillin
1	A38a+ca	5.7	6.25	11.4	12.5
2	A31b+	5.7	6.25	5.7	6.25
3	A8a+ca	22.8	12.5	22.8	12.5
4	A5a1+	11.4	6.25	22.8	12.5
5	A66a+	11.4	12.5	22.8	12.5
6	C31b+	5.7	6.25	5.7	12.5
7	A31b2+	5.7	12.5	11.4	12.5
8	A11c+ca	11.4	6.25	11.4	12.5
9	A22a+ca	5.7	12.5	22.8	12.5
10	A1c	2.85	6.25	5.7	12.5
11	A11b+ca	2.85	6.25	5.7	12.5
12	A22a+	5.7	12.5	11.4	12.5
13	B31d+	5.7	12.5	22.8	12.5
14	A5a2+	5.7	0.781	11.4	1.563
15	A8a+	5.7	3.125	5.7	12.5
16	A3a+	22.8	6.25	22.8	6.25
17	A10b+	5.7	12.5	11.4	12.5
18	A22a1+ca	2.85	1.563	5.7	3.125
19	B34a+	2.85	6.25	11.4	12.5
20	A26b+	1.43	1.563	2.85	6.25

Table 2. Profile of MIC and MBC of cephalosporin for *Staphylococcus aureus* isolates

S/N	Isolates code	MIC for cephalosporin (mg/mL)			MBC for cephalosporin (mg/mL)		
		Cephalexin	Cefuroxime	Ceftazidime	Cephalexin	Cefuroxime	Ceftazidime
1	A38a+ca	6.25	6.25	0.781	12.5	12.5	3.125
2	A31b+	12.5	6.25	3.125	12.5	12.5	6.25
3	A8a+ca	6.25	6.25	6.25	12.5	12.5	12.5
4	A5a1+	6.25	6.25	6.25	12.5	12.5	12.5
5	A66a+	6.25	12.5	3.125	12.5	12.5	6.25
6	C31b+	6.25	6.25	1.563	6.25	12.5	6.25
7	A31b2+	3.125	6.25	6.25	12.5	12.5	12.5
8	A11c+ca	6.25	12.5	6.25	12.5	12.5	12.5
9	A22a+ca	12.5	3.125	12.5	12.5	12.5	12.5
10	A1c	6.25	6.25	6.25	12.5	12.5	12.5
11	A11b+ca	6.25	12.5	6.25	12.5	12.5	6.25
12	A22a+	12.5	6.25	3.125	12.5	12.5	12.5
13	B31d+	6.25	12.5	0.781	12.5	12.5	3.125
14	A5a2+	3.125	6.25	3.125	12.5	12.5	6.25
15	A8a+	6.25	3.125	1.563	12.5	12.5	1.563
16	A3a+	12.5	6.25	6.25	12.5	12.5	6.25
17	A10b+	6.25	3.125	1.563	12.5	12.5	12.5
18	A22a1+ca	6.25	6.25	1.563	12.5	12.5	3.125
19	B34a+	3.125	12.5	1.563	12.5	12.5	12.5
20	A26b+	12.5	12.5	0.781	12.5	12.5	0.781

Table 3. Profile of MIC and MBC of ciprofloxacin for *Staphylococcus aureus* isolates

S/N	Isolates code	MIC for ciprofloxacin (mg/mL)	MBC for ciprofloxacin (mg/mL)
1	A38a+ca	0.781	1.563
2	A31b+	0.781	1.563
3	A8a+ca	0.781	1.563
4	A5a ₁ +	0.391	0.781
5	A66a+	0.195	0.391
6	C31b+	0.391	0.781
7	A31b ₂ +	0.781	1.563
8	A11a+ca	0.391	0.781
9	A22a+ca	0.195	0.391
10	A1c	0.391	0.781
11	A11b+ca	0.781	0.781
12	A22a+	0.781	1.563
13	B31d+	0.781	1.563
14	A5a ₂ +	0.391	3.125
15	A8a+	0.781	3.125
16	A3a+	0.391	0.781
17	A10b+	0.781	3.125
18	A22a ₁ +ca	0.195	0.391
19	B34a+	0.391	0.781
20	A26b	0.391	0.781

Table 4. Profile of MIC and MBC of antibiotics that inhibit translation for *Staphylococcus aureus* isolates

S/N	Isolatels code	MIC (mg/mL)				MBC (mg/mL)			
		Gentamicin	Erythromycin	Tetracycline	Chloramphenicol	Gentamicin	Erythromycin	Tetracycline	Chloramphenicol
1	A38a+ca	0.008	0.008	0.004	0.016	0.016	0.016	0.008	0.032
2	A31b+	0.008	0.016	0.008	0.016	0.008	0.016	0.008	0.032
3	A8a+ca	0.016	0.008	0.004	0.016	0.016	0.016	0.008	0.032
4	A5a1+	0.016	0.016	0.004	0.016	0.016	0.016	0.008	0.032
5	A66a+	0.008	0.016	0.008	0.016	0.016	0.016	0.008	0.032
6	C31b+	0.008	0.008	0.004	0.016	0.016	0.016	0.008	0.032
7	A31b2+	0.008	0.008	0.004	0.016	0.008	0.016	0.008	0.032
8	A11c+ca	0.008	0.008	0.004	0.016	0.016	0.016	0.008	0.032
9	A22a+ca	0.016	0.008	0.004	0.016	0.016	0.016	0.008	0.032
10	A1c	0.016	0.008	0.004	0.032	0.016	0.016	0.008	0.032
11	A11b+ca	0.008	0.016	0.008	0.016	0.016	0.016	0.008	0.032
12	A22a+	0.016	0.008	0.008	0.032	0.016	0.016	0.008	0.032
13	B31d+	0.008	0.008	0.004	0.016	0.008	0.016	0.008	0.032
14	A5a2+	0.008	0.008	0.008	0.016	0.016	0.016	0.008	0.032
15	A8a+	0.008	0.008	0.004	0.032	0.008	0.016	0.004	0.032
16	A3a+	0.008	0.008	0.004	0.016	0.008	0.016	0.008	0.032
17	A10b+	0.016	0.008	0.004	0.016	0.016	0.016	0.008	0.032
18	A22a1+ca	0.016	0.008	0.008	0.016	0.016	0.016	0.008	0.032
19	B34a+	0.008	0.008	0.004	0.016	0.008	0.016	0.008	0.032
20	A26b+	0.008	0.016	0.004	0.032	0.016	0.016	0.008	0.032

Table 5. Profile of MIC and MBC of penicillin for *Escherichia coli* isolates

S/N	Isolates code	MIC for penicillin (mg/mL)		MBC for penicillin (mg/mL)	
		Augmentin	Amoxicillin	Augmentin	Amoxicillin
1	A39b+	11.4	6.25	22.8	12.5
2	A43a+	5.7	3.125	11.4	6.25
3	A5b+	11.4	6.25	22.8	12.5
4	A31c+	11.4	6.25	11.4	12.5
5	A40a+	1.43	6.25	5.7	12.5
6	A33a+	11.4	6.25	22.8	12.5
7	A20a+mac	11.4	6.25	22.8	12.5
8	A34a+ca	11.4	1.563	22.8	3.125
9	A2a+	5.7	6.25	22.8	12.5
10	A34a+	11.4	6.25	11.4	12.5

Table 6. Profile of MIC and MBC of cephalosporin for *Escherichia coli* isolates

S/N	Isolates code	MIC for cephalosporin (mg/mL)			MBC for cephalosporin (mg/mL)		
		Cephalexin	Cefuroxime	ceftazidime	Cephalexin	Cefuroxime	Ceftazidime
1	A39b+	1.563	3.125	1.563	3.125	6.25	6.25
2	A43a+	6.25	6.25	0.781	6.25	6.25	1.563
3	A5b+	3.125	6.25	0.196	6.25	12.5	0.781
4	A31c+	6.25	6.25	0.781	12.5	12.5	1.563
5	A40a+	6.25	3.125	0.391	12.5	6.25	3.125
6	A33a+	6.25	6.25	0.391	12.5	12.5	3.125
7	A20a+mac	3.125	6.25	1.563	6.25	12.5	6.25
8	A34a+ca	6.25	3.125	0.781	12.5	6.25	0.781
9	A2a+	6.25	6.25	0.781	12.5	12.5	6.25
10	A34a+	3.125	3.125	0.781	6.25	12.5	1.563

Table 7. Profile of MIC and MBC of ciprofloxacin for *Escherichia coli* isolates

S/N	Isolates code	MIC for ciprofloxacin (mg/mL)	MBC ciprofloxacin (mg/mL)
1	A39b+	0.781	1.563
2	A43a+	1.563	3.125
3	A5b+	3.125	3.125
4	A31c+	0.391	0.781
5	A40a+	3.125	3.125
6	A33a+	1.563	3.125
7	A20a+mac	0.781	1.563
8	A34a+ca	0.781	1.563
9	A2a+	1.563	1.563
10	A34a+	1.563	3.125

4. DISCUSSION AND CONCLUSION

4.1 Discussion

The emergence of resistant bacteria is due to misuse and overuse of antibiotics as well as inappropriate prescription by the clinicians [13]. Antibiotic resistance has become a major clinical and public health problem within our lifetime. The study evaluates the antibiotic resistance profile of bacterial isolates, determined the minimum inhibitory concentration

(MIC) and minimum bactericidal concentration (MBC) values of antibiotics common used in this environment against isolates from HIV seropositive. The MIC and MBC were determined for each bacterial isolate. The study showed that the MIC values of the antibiotics tested against *S. aureus*, *E. coli* and *Pseudomonasa eruginosa* and *Pseudomonas fluorescens* which were the predominant bacterial isolates cultured from urine samples of HIV seropositive pregnant women were high. These bacterial isolates were multiple resistant

Table 8. Profile of MIC and MBC of antibiotics that inhibit translation for *Escherichia coli* isolates

S/N	Isolates code	MIC (mg/mL)				MBC (mg/mL)			
		Gentamicin	Erythromycin	Chloramphenicol	Tetracycline	Gentamicin	Erythromycin	Chloramphenicol	Tetracycline
1	A39b+	0.008	0.008	0.008	0.004	0.016	0.008	0.016	0.008
2	A43a+	0.016	0.008	0.008	0.008	0.016	0.016	0.008	0.008
3	A5b+	0.008	0.016	0.016	0.004	0.016	0.016	0.016	0.008
4	A31c+	0.008	0.016	0.008	0.008	0.016	0.016	0.016	0.008
5	A40a+	0.016	0.008	0.016	0.004	0.016	0.016	0.016	0.008
6	A33a+	0.008	0.008	0.008	0.008	0.008	0.008	0.016	0.008
7	A20a+mac	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
8	A34a+ca	0.008	0.008	0.008	0.004	0.016	0.016	0.016	0.008
9	A2a+	0.008	0.008	0.016	0.008	0.008	0.016	0.016	0.008
10	A34a+	0.008	0.016	0.008	0.004	0.016	0.016	0.016	0.008

Table 9. Profile of MIC and MBC of penicillin for *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*

S/N	Isolates code	MIC for penicillin (mg/mL)		MBC for penicillin (mg/mL)	
		Augmentin	Amoxicillin	Augmentin	Amoxicillin
1	A37a+	2.85	-	5.7	-
2	B22c+	11.4	-	11.4	-
3	A16a+ca	2.85	-	11.4	-
4	B17b+	2.85	-	5.7	-
5	A17a+ca	2.85	-	5.7	-
6	A35a+	2.85	-	2.85	-
7	A29a+mac	5.7	-	11.4	-
8	A100a+ca	11.4	-	11.4	-
9	B34b+	5.7	-	11.4	-
10	A38b+mac	2.85	-	5.7	-
11	A2a+mac	5.7	-	11.4	-
12	A22a+ca	5.7	-	5.7	-
13	A30e+	2.85	-	5.7	-
14	A34c+	5.7	-	5.7	-
15	B4c+	2.85	-	11.4	-

S/N	Isolates code	MIC for penicillin (mg/mL)		MBC for penicillin (mg/mL)	
		Augmentin	Amoxicillin	Augmentin	Amoxicillin
16	A12b+	2.85	-	5.7	-
17	A30e+ca	2.85	-	5.7	-
18	A1ba+ca	11.4	-	11.4	-
19	C35b+	2.85	-	5.7	-
20	A16a+	2.85	-	11.4	-

Table 10. Profile of MIC and MBC of cephalosporin for *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* isolates

S/N	Isolates code	MIC for cephalosporin (mg/mL)				MBC for cephalosporin (mg/mL)			
		Cephalexin	Cefuroxime	Cefoxitin	Ceftazidime	Cephalexin	Cefuroxime	Cefoxitin	Ceftazidime
1	A37a+	-	-	-	3.125	-	-	-	6.25
2	B22c+	-	-	-	1.563	-	-	-	6.25
3	A16a+ca	-	-	-	3.125	-	-	-	12.5
4	B17b+	-	-	-	3.125	-	-	-	6.25
5	A17a+ca	-	-	-	3.125	-	-	-	6.25
6	A35a+	-	-	-	1.563	-	-	-	12.5
7	A29a+mac	-	-	-	6.25	-	-	-	12.5
8	A100a+ca	-	-	-	6.25	-	-	-	12.5
9	B34b+	-	-	-	6.25	-	-	-	12.5
10	A38b+mac	-	-	-	3.125	-	-	-	12.5
11	A2a+mac	-	-	-	3.125	-	-	-	3.125
12	A22a+ca	-	-	-	1.563	-	-	-	6.25
13	A30e+	-	-	-	6.25	-	-	-	12.5
14	A34c+	-	-	-	3.125	-	-	-	12.5
15	B4c+	-	-	-	1.563	-	-	-	12.5
16	A12b+	-	-	-	6.25	-	-	-	12.5
17	A30e+ca	-	-	-	6.25	-	-	-	12.5
18	A1ba+ca	-	-	-	6.25	-	-	-	12.5
19	C35b+	-	-	-	3.125	-	-	-	12.5
20	A16a+	-	-	-	3.125	-	-	-	12.5

Table 11. Profile of MIC and MBC of ciprofloxacin for *Pseudomonas aeruginosa* and *fluorescens* isolates

S/N	Isolates code	MIC for ciprofloxacin (mg/mL)	MBC for ciprofloxacin (mg/mL)
1	A37a+	1.563	3.125
2	B22c+	1.563	3.125
3	A16a+ca	0.391	3.125
4	B17b+	0.782	1.563
5	A17a+ca	0.782	3.125
6	A35a+	0.782	1.563
7	A29a+mac	1.563	1.563
8	A100a+ca	1.563	3.125
9	B34b+	1.563	1.563
10	A38b+mac	0.782	3.125
11	A2a+mac	3.125	3.125
12	A22a+ca	3.125	3.125
13	A30e+	1.563	3.125
14	A34c+	1.563	3.125
15	B4c+	3.125	3.125
16	A12b+	0.782	3.125
17	A30e+ca	3.125	3.125
18	A1ba+ca	1.563	3.125
19	C35b+	1.563	3.125
20	A16a+	0.391	0.781

to antibiotic to which they tested. The MIC and MBC values obtained for augmentin was found to be more effective in *S. aureus* (22.8 mg/mL) and moderately effective against *E. coli* (1.43/2.85 mg/mL). The MIC and MBC values of all the antibiotics used against *S. aureus* isolates range from 0.004/0.008 mg/mL to 22.8 mg/mL.

The MIC and MBC values of all the antibiotics tested against *E. coli* isolates ranged from 0.008/0.016 mg/mL to 11.4/22.8 mg/mL, while the MIC and MBC values of all the antibiotics tested against *Pseudomonas* spp range from 0.391/0.781 mg/mL to 11.4/12.5 mg/mL. Also, the MIC values of all the antibiotics used against *E. coli* and *Pseudomonas* spp may be considered moderate when compared with previous studies in Dhaka [12,14].

Beta-lactams antibiotics are the most commonly used antibiotics world -wide which make them prone to misuse and abuse thereby, leading to problem of resistance. This study revealed the effectiveness of each antibiotic under the cephalosporin group, where the third generation of cephalosporin was more effective and relevant to some extent in the cause of bacterial infections treatment compared to the second generation and the first generation of cephalosporin as well as the penicillin group.

However, some of the antibiotics such as gentamicin, erythromycin, tetracycline and

chloramphenicol, showed moderate effect against these bacterial isolates after the antibiotics were double strength. The MIC and MBC of ciprofloxacin used against the bacterial isolates involved in this study ranged from 0.195/0.781 mg/mL to 3.125 mg/mL. Of all the antibiotics used, ciprofloxacin was observed to be more effective against the bacterial isolates. Studies done by Kowser and Fetema [12] in Bangladesh on *S. aureus*, *Pseudomonas* spp. *E. coli* and *Shigella* spp from clinical sources reported that ciprofloxacin was more effective than other antibiotics used against these bacterial isolates [12].

4.2 Conclusion

Most of the bacterial isolates used for this study were multi-resistant to different antibiotics tested *in vitro* suggesting the prevalence of multiple resistance strains among immune compromised patients which is worrisome. The effectiveness comparison among beta lactams class of antibiotic showed that cephalosporin third generation was more effective and relevant to some extent in the cause of bacterial infections treatment compared to the second generation and first generation as well as the penicillin group. In this regard, clinicians should sought additional therapies to supplement the current standard of care and also control use of the new drug.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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