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Original Research Article

ANTIBIOTIC RESISTANCE PATTERN OF ACINETOBACTER BAUMANNII ISOLATES FROM VARIOUS CLINICAL SPECIMENS

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Abstract

Background: Acinetobacter baumannii is a gram-negative coccobacillus that has been listed as the most important pathogen in hospitals. A. baumannii is an opportunistic pathogen with a wide spectrum, and it has been described as a dangerous pathogen by the Infectious Disease Society of America Here, we hypothesize that the use of antibiotics in clinical settings may affect the evolution of bacterial resistance to phages. Consequently, we used clinically isolated A. baumannii strains as models to demonstrate the correlation between bacterial resistance to antibiotics. **Materials and Methods:** A total of 544 samples were collected from patients in different services (reanimation, paediatrics and Medicine) and the outpatient department at the Department of Microbiology, BMIMS, Pawapuri, and Nalanda from September 2022 to February 2023. Specimens analyzed included blood (112), catheter (24), pus (198) and pulmonary (210). All collected samples were cultured on blood and chocolate agar by the 4-quadrant streak plate method [9]. After 18 h of incubation (at 35 °C), pure cultures of presumptive A. baumannii were first subjected to the standard bacteriological techniques (morphology, Gram stain, catalase test and oxidase test, and identified later as A. baumannii. Antibacterial Resistance Study Antibacterial resistance of A. baumannii strains was determined for 11 antibiotics by the disk diffusion method. Sensitivity or resistance of each strain to the antibacterial drugs was determined according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI). **Result:** Some strains were total resistant to all the 11 antibiotics tested while others were highly resistant [Table 1]. Our results showed that all A. baumannii strains were resistant to all β-lactams, except for 18.08% strains that were sensitive to imipenem. **Conclusion:** Thus, faced with this A. baumannii antibacterial resistance difficulty and a limited number of active molecules against it, Actinobacteria could be an alternative source of bioactive molecules that could meet this increased necessity.

INTRODUCTION

Acinetobacter baumannii is a gram-negative coccobacillus that has been listed as the most important pathogen in hospitals.[1] A. baumannii is an opportunistic pathogen with a wide spectrum, and it has been described as a dangerous pathogen by the Infectious Disease Society of America.[2] Following the introduction of antibiotics into clinical use, A. baumannii has become problematic in intensive care units (ICU) because it has developed resistance to broad-spectrum antibiotics,[3] and the incidence of multidrug-resistant A. baumannii (MDRAB) infections has continued to increase worldwide.[4]

Because of the rapid accumulation of resistance to multiple antibiotics, carbapenems are the only effective group of antibiotics against MDRAB infections. Carbapenems are classified as a subgroup of the β-lactam antibiotics. Similar to other β-lactams, carbapenems can bind to the penicillin-binding proteins of bacteria, which inhibit cell wall synthesis and results in cell death1. Unfortunately, the number of A. baumannii strains showing resistance to carbapenems has been increasing rapidly.[5] Although last resort treatments are available for MDRAB, such as colistin,[6] these antibiotics may cause severe physical side effects, including neurotoxicity or nephrotoxicity.[6]

Bacteriophage (phage) therapy represents a potential alternative strategy for treating infections with multidrug-resistant (MDR) strains. Phages are host-specific, natural parasites of bacteria. Because

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antibiotic-resistant bacteria are a serious health concern, the use of phage's to reduce the concentration of specific bacterial pathogens has experienced renewed interest.[7] The first active phage shown to specifically infect MDRAB was characterized in 2010,[8] and several isolated phage strains capable of infecting MDRAB have subsequently been identified,[9-11] Our group also isolated A. baumannii-specific phage's for different applications.[8,12-15]

Twenty-four new phage strains have been isolated by our group, and we expect that these phage's have the potential for use as bio control agents,[16] and could be applied in clinical and environmental settings.[15] Before applying these phage's for therapy or environmental cleaning, the susceptibility of clinically isolated A. baumannii strains to these 24 phages should be determined. The development of resistance to different antibiotics may be correlated with the evolution of resistance to phages.[17] This antagonistic co evolutionary relationship among bacteria, antibiotics and phage's must be investigated via studies focused on clinical and environmental settings. Although in vitro tests have been conducted to explore the cross-resistance profile to antibiotics and phages using a single bacterial strain,[17-19] the cross-resistance profiles of clinical bacteria to multiple antibiotics and environmental phages has not been studied.

Here, we hypothesize that the use of antibiotics in clinical settings may affect the evolution of bacterial resistance to phages. Consequently, we used clinically isolated A. baumannii strains as models to demonstrate the correlation between bacterial resistance to antibiotics

MATERIALS AND METHODS

A total of 544 samples were collected from patients in different services (reanimation, paediatrics and Medicine) and the outpatient department at the Department of Microbiology, BMIMS, Pawapuri, and Nalanda from September 2022 to February 2023. Specimens analyzed included blood (112), catheter (24), pus (198) and pulmonary (210).

All collected samples were cultured on blood and chocolate agar by the 4-quadrant streak plate method [9]. After 18h of incubation (at 35°C), pure cultures of presumptive A. baumannii were first subjected to the standard bacteriological techniques (morphology, Gram stain, catalase test and oxidase test, and identified later as A. baumannii conventional tests which include potassium nitrate (NO3), l-tryptophane (TRP), d-glucose (GLU), l-arginine (ADH), urea (URE), esculin ferric citrate (ESC), gelatin (GEL) and 4-nitrophenyl-β-D-galactopyranoside (PNPG) and also assimilation of 12 compounds, which include d-glucose (GLU), L-arabinose (ARA), d-mannose (MNE), D-mannitol (MAN), N-acetyl-glucosamine (NAG), d-maltose (MAL), potassium gluconate (GNT), capric acid (CAP), adipic acid (ADI), malic acid (MLT), trisodium citrate (CIT) and phenyl acetic acid (PAC). Wells of biochemical test were inoculated with 0.5 McFarland bacterial suspensions and incubated for 48 h (at 37 °C).

Antibacterial Resistance Study Antibacterial resistance of A. baumannii strains was determined for 11 antibiotics by the disk diffusion method. Sensitivity or resistance of each strain to the antibacterial drugs was determined according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI) [13]. Antibiotics tested were ticarcillin (75 µg), piperacillin (100µg), ticarcillin + clavulanic acid (75/10µg), ceftazidime (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (cotrimoxazole: 1.25/23.75 µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg) and doxycycline (30 µg). Minimum inhibitory concentration (MIC) of colistin (polymyxin E) was determined by agar dilution method according to the recommendations of CLSI. Briefly, Mueller Hinton agar (MHA) containing different concentrations of colistin which ranged from 0.125 to 256 µg/ml was inoculated with a bacterial suspension whose turbidity was adjusted to 0.5 McFarland.

RESULTS

Macroscopic examination of cultures showed smooth, circular, convex and whitish colonies. Microscopic examination after Gram differential staining revealed small bacilli or coccobacillus bacteria, with mixed (variable) staining (mainly pink and purple), rounded ends, isolated or grouped in two or in short chains. Enzymatic tests performed on the bacterial colonies presumptively identified as Acinetobacter (basing on morphological characterization and Gram stain), revealed a negative reaction to oxidase (oxidase +), positive reaction to catalase (catalase +) and oxidative degradation of sugars on MEVAG medium. All bacterial strains with the above characteristics were identified by API 20 NE (based on biochemical tests), which is widely used in hospital laboratories. The antibiotic resistance profile of A. baumannii strains was determined using the disk diffusion technique. Some strains were total resistant to all the 11 antibiotics tested while others were highly resistant [Table 1]. Our results showed that all A. baumannii strains were resistant to all β-lactams, except for 18.08% strains that were sensitive to imipenem.

Table 1: Antibiotic resistance profile of A. baumannii

A. baumannii strains	TIC	TCC	PIP	CAZ	IMP	AK		GN	TM	DO	SXT	CIP	CL
	R	R	R	R	R	R		R	R	R	R	R	S

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	R	R	R	R	R	R		S	R	R	S	S	S
	R	R	R	R	R	S		S	R	S	S	R	S
	R	R	R	R	R	R		R	R	R	S	S	S
	R	R	R	R	S	R		R	R	R	S	R	S
	R	R	R	R	S	R		R	R	R	S	S	S
	R	R	R	R	R	S		R	R	S	S	R	S

B-lactams: (TIC ticarcillin, TCC ticarcillin + clavulanic acid, PIP piperacillin, CAZ ceftazidime, IMP imipenem), aminosides: (AK amikacin, GN gentamicin, TM tobramycin), DO doxycycline, SXT cotrimoxazole, CIP ciprofloxacin, CL colistin, R resistant, S sensible

DISCUSSION

In our study, the resistance of A. baumannii to aminoglycosides was recorded as 100% for tobramycin and exceeded 85% for gentamicin. These resistance rates were high compared to those found in Europe, which were 47.1% for gentamicin,[20] and 41% for tobramycin.[13] Concerning the resistance to ciprofloxacin, a rate of 86.36% was noted in our study, which was close to that recently stated in Morocco (91%).[14] these results allowed us to consider our strains as MDR (multidrug resistant).[15] Lastly, all strains were sensitive to colistin with MICs not exceeding 1 µg/ml. Our results were similar to those obtained by Kandeel,[16] who stated the MIC of colistin as less than 2 µg/ml.

The antibiogram complimentary tests phenotypic ally revealed three enzymatic mechanisms of resistance in the tested A. Baumannii strains: extended-spectrum-lactamases (ESBL), cephalosporins and metallo-β-carbapenemase.

Only two strains (14.97%), out of 17 tested, were ESBL producers. According to the national surveillance for resistant bacteria, the production of ESBL was 22.02% in 16 tested laboratories which show that the number of A. baumannii ESBL producers remains low.[17] Furthermore, screening of these strains for cephalosporinase revealed that 10 strains (52.63%) of A. baumannii were cephalosporinase positive. Yongrui and Xiangqun,[18] revealed a higher rate (72%). The phenotypic tests EDTA and Hodge highlighted the production of metallo β-carbapenemase by 15 strains (78.95%), similar to a rate that was found by Kaur et al. (80.3%). On the other hand, lower rates (between 22 and 49%) of A. baumannii metallo β-lactamase positive strains were reported by many authors.[21,22]

Fifty-seven actinobacterial isolates cultured on ISP2 medium were tested against 19 A. baumannii strains by agar plug diffusion method to evaluate their antibacterial potential. The antibacterial activity screening revealed that 17 (30.10%) isolates were active against at least one A. baumannii strain. In contrast, 50 (68.70%) isolates showed no activity. An antibacterial activity was obtained by all 13 Saccharothrix (or Saccharothrix-like) isolates, followed by five Actinomadura isolates and one isolate of Streptomyces. The antagonistic activity against A. baumannii obtained in the 17 active isolates was generally strong for the majority of Saccharothrix (or Saccharothrix-like) isolates and the single Streptomyces isolate (WAB9). It was average for the three Actinomadura isolates. The diameters of inhibitions obtained by these active isolates varied between 10 and 30 mm. We noticed a variation in diameters of inhibition between isolates of the same genus; those of Saccharothrix (or Saccharothrix-like) ranged from 11 to 30 mm, whereas those of Actinomadura ranged from 10 to 22 mm. The Saccharothrix isolates were highly active with a maximum inhibition diameter of 30 mm against some A. baumannii strains. In contrast, in the scientific literature, most antibiotics secreted by Saccharothrix isolates mainly target Gram-positive bacteria and fungi, and are rarely directed against Gram-negative bacteria.[13–17]

Secondly, the Streptomyces sp. WAB9 followed with a maximum inhibition zone of 25 mm. Our results were similar to those of Yekkour et al,[18] who stated that this strain of Streptomyces sp. has a broad spectrum that extends over several filamentous fungi, and a large number of pathogenic bacteria (Gram-positive and Gram-negative), which are multi-resistant to several antibiotics. These authors found that the high activity of Streptomyces WAB9 (close to the species Streptomyces ambofaciens) is due to the production of a new antibiotic (named W9), which is a hydroxamic acid-containing molecule. Hydroxamic acid containing molecules are known to exhibit low toxicities in general, which is very interesting. Finally, Actinomadura was active with a maximum inhibition zone of 22 mm. Actinomadura isolates are known for the production of several antibacterial molecules, namely maduramicin, actinotiocin and carminomycin secreted, respectively, by A. rubra, A. pusilla and A. carminata.[19] The action spectrum of the antibacterial molecules secreted by the active isolates was quite broad for the majority of isolates with a maximal spectrum obtained by Streptomyces sp. WAB9.

CONCLUSION

The majority of the strains were isolated from pulmonary samples received from patients hospitalized in the intensive care unit. The evaluation of antibiotic resistance of these strains against antibiotics recommended for treatment of A. baumannii infections revealed the ineffectiveness of the majority of the molecules. We noted a total absence of β-lactam antibacterial activity against all the strains. This was attributed to the production of different enzymes that degraded the antibiotic molecules namely metallo-β-carbapenemase and

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cephalosporinase. Nonetheless, all the A. baumannii strains were sensitive to colistin which was active at very low MICs. The results from the antagonistic potential evaluation carried out on a collection of Actinobacteria showed the ability of certain isolates belonging to the genera Saccharothrix, Streptomyces and Actinomadura to secrete bioactive molecules against A. baumannii. Thus, faced with this A. baumannii antibacterial resistance difficulty and a limited number of active molecules against it, Actinobacteria could be an alternative source of bioactive molecules that could meet this increased necessity.

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