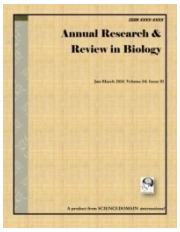

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The Antibacterial Activity of 2-aminoindanbiguanide Chloride and Acetate Salts against Resistant Gram-negative Bacilli

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Authors' contributions

Authors HG and OK designed the study. Both authors have searched literature for their section. Author HG synthesized this molecules and author OK studied this molecules against MDR-GNB. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Antiseptics and disinfectants are important substances for medical care. However, the needs for new antiseptics have increased in the last few years, due to multi-drug resistant pathogens. In this study we aimed to evaluate, the efficacy of 2-aminoindanbiguanide chloride (2-AIB.HCl) and 2-aminoindanbiguanideacetate (2-AIB.HOAc) salts that were synthesized against multi-drug resistant Gram-negative bacilli (MDR-GNB) (*Acinetobacter spp*, *Escherichia coli* and *Klebsiella pneumoniae*). According to our resultsat70 mg/mL concentrations of 2-AIB.HCl and 2-AIB.HOAc salts have been confirmed to be effective against hospital infection causing MDR-GNB. It was shown that 2-AIB.HCl and 2-AIB.HOAc salts have antibacterial efficacy against MDR-GNB.

Keywords: Multi-drugresistantbacteria; disinfectant; disinfection; 2-aminoindanbiguanide salts.

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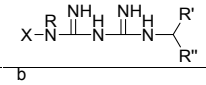
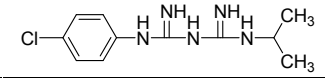
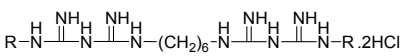
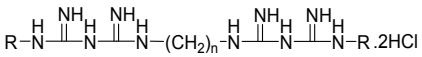
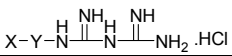
1. INTRODUCTION

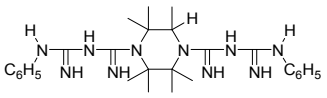
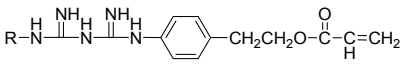
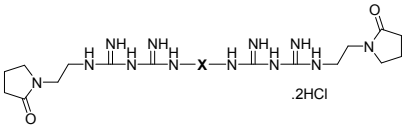
Biguanidine derivatives are widely used in medical care. These compounds are used for disinfectant purposes [1-5]. Moreover, these compounds are of anti parasitic nature [6,7]. Beside their common use in hospitals, disinfectant substances are also commonly used in dentistry, food sector and houses/kitchens. Disinfectants are one of the main agents of the efforts that aim to prevent cross infections. The widespread use of disinfectants causes the MDR-GNB. Due to multiple drug resistance problem of treatment of some bacteriaı infections, there is need for new effective products to tackle this problem.

Polyhexamethylene biguanide (PHMB) is a biguanide derivative that is used as an antiseptic in the treatment and prevention of infection. With the discovery of the biological aspects of PHMB, the synthesis of biguanide compounds and their medical use came into prominence [8].

Many biguanide derivatives have been synthesized so far, some of which are used as antiseptics (Table 1). However, no research regarding the antiseptic efficacy of 2-aminoindanbiguanide chloride (2-AIB.HCl) and 2-aminoindanbiguanide acetate (2-AIB.HOAc) salts on MDR-GNB has been reported. The efficacy of 2-AIB salts against multi-drug resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* were investigated for the first time with this study.

Table 1. Some reported biguanide derivatives with effective activity against microorganisms

Researcher	Year	Test organism(s)/ effect	Molecule Structures
F.H.S. Curd	1946	Anti-parasitic [9]	a 
P. Ray	1961	Anti- parasitic [10]	b 
R.A. Cutler	1964	*Staphylococcus aureus* *Salmonella typhi* *Clostridium spp.* *Pseudomonas aeruginosa* *Tinea mentagraphytes* *Aspergillus niger* *Candida albicans* [11]	
R.A. Coburn	1978	*S. mutants* *A. naesludii* *A. viscosus* [12]	c 
N.Ishada	1965	Antiviral [13]	d 

J.W. James	1968	*Staphylococcus aureus* *Escherichia coli* *Pseudomonas pyocyaneus* *Salmonella* [14]	
T. Ikeda	1984	*Bacillus subtilis* *Staphylococcus aureus* *Escherichia coli* *Aerobacter aerogenes* *Pseudomonas aeruginosa* [15]	[e] 
J.J. Merianos	1992	*Escherichia coli* *Pseudomonas aeruginosa* *Streptococcus pyogenes* *Staphylococcus aureus* Fungi [16]	[f] 

[a]X: aryl (p-chlorophenyl, phenyl, 3,4-dimethyl); [b]Paludrine; [c] n: 1-10; [d] X: an amino group and Y: a CO-Aryl or SO_2-Aryl Group; [e] R: phenyl, p-Chlorophenyl, 3,4-dichloropheniyl; [f] X:hexane,dodecane, etoxyethane

2. MATERIALS AND METHODS

2.1 MDR-GNB Bacteria Definition

Multi-drug resistance among Gram-negative bacilli was defined as resistance to three or more of the following antimicrobials: meropenem, gentamicin, imipenem, ceftazidime, ciprofloxacin, ampicillin/sulbactam, or piperacillin/tazobactam [17].

2.2 Samples and Sources

The hospital infection definition was made according to CDC (Center for Disease Control and Prevention, Atlanta, USA) definitions. During the hospital infection surveillance various culture samples (blood, urine, sputum, etc.) were collected. All bacteria identification was made with classical microbiological methods. Antibiotic sensitivity tests were performed by using the disc diffusion method according to CLSI (Clinical and Laboratory Standards Institute) criteria. The existence of ESBL was investigated using a double-disc synergy method on Mueller-Hinton agar [18].All of Gram-negative bacilli were acquired from hospital infections which are caused by bacteria, stored in Sakarya University Hospital Department of Infectious Disease and Microbiology Laboratory.

2.3 Antibacterial Studies

The bacteria used in our trial were obtained from patient samples of intensive care units (ICU) of Sakarya University Training and Research Hospital between 2008 and 2013. These bacteria caused bacteremia, cross infection causing pneumonia or urinary infections acquired during hospital stay. The derived bacteria were formed out of Gram-negative bacteria that were identified to be resistant against at least 3 different sets of antibiotics (wide spectrum penicillin's aminoglycosides, carbapenems and/or quinolones). The bacteria was named through classical biochemical methods and Vitek2 (bioMerieux) full automatic

determination system. Blood cultures were followed up in full automatic blood culture device (Biomerieoux, France). Positive and negative controls were used for antimicrobial test. Sterile saline was used for negative control and *E. coli* ATCC 25922 was used for positive control.

2.2 The Synthesis of 2-aminoindanbiguanide Chloride and Acetate Salts

Synthesis of 2-AIB.HCl salt: 2-AIB.HCl salt (1.5 g, 4.98mol) and Dicyandiamide (0.418 g, 4.98mol) were swirled at 160ºC for 100 minutes. The mixture initially melted and then solidified. Afterwards, it was allowed to cool down to room temperature. The acquired solid substance was re-crystallized over the EtOH/ Et_2O (4:3) mixture, and 2-AIB.HCl salt was acquired with an efficiency of 50% in beige crystal condition [19].

Synthesis of 2-AIB.HOAc salt: 2-AIB-HCl salt (2 g) was dissolved in water (40 ml) and was neutralized with a 10%NaOH solution. It was extracted twice through ethyl acetate. The combined organic phases were dried through $MgSO_4$ and ethyl acetate was suspended in the evaporator. The remnant was dissolved in ethanol and treated with the glacial acetic acid surplus. The sinking solid was glided and dried. 2-AIB.HOAc salt was acquired with an efficiency of 42% (Fig. 1).

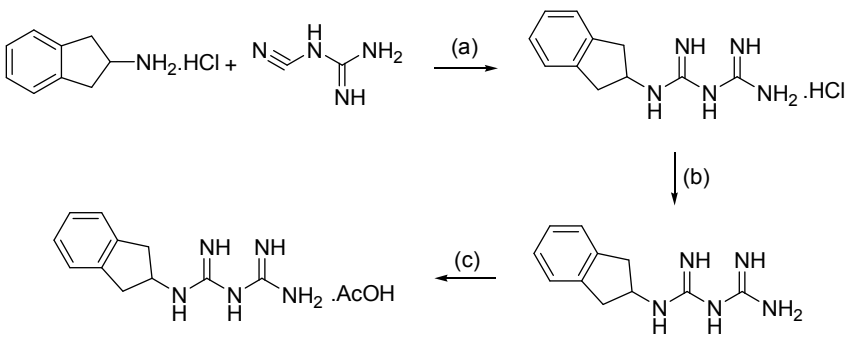


Fig. 1. Reagents and conditions: (a) No Solvent, 160ºC, 100 min; (b) 10%NaOH in water, (c) AcOH (Glacial), EtOH

3. RESULTS AND DISCUSSION

Eighty-four (84) resistant bacteria were included in our study (21 *Acinetobacter baumannii*, 59*Escherichia coli*, 5*Klebsieallapneumoniae*). The mean antibacterial sensitivity is summarized in Table 2.

The zone diameters and sensitivity of the 2-AIB ESBL (+) salts against *E.coli* (Fig. 2), *Acinetobacter baumannii* (Fig. 3) and *Klebsiella pneumoniae* (Fig. 4) are shown in graphs.

A wide variety of biological activities of biguanide derivatives were known for many years [1]. At the present time some of biguanide derivatives such as met for min (anti diabetic drug), proguanil (anti malarial drug), polyhexamethylene biguanide (antiseptic), chlorhexidine (antiseptic) are used commercially. Recent studies about biguanide aimed at discovering of alternative medicines or improving the effectiveness of these drugs [20-22]. For example, according to a study which is recently declared, alexidine has greater affinity for bacterial

virulence factors than more commonly used chlorhexidine [23]. Studies for the development of anticancer pharmaceuticals about met for min which is also an anti diabetic drug are remarkable [24-26]. Newly synthesized chitosan biguanidine hydrochloride salt is investigated on application in antimicrobial finish of wool fabric [27].

Table 2. The mean inhibition zone diameter (in mm) of anti bacterialsu sceptibility of 2-AIB salts

	ESBL *(+) E. coli* (mean zone diameter, mm)	***Klebsiella pnemoniae* (mean zone diameter, mm)**	***Acinetobacterb aumannii* (mean zone diameter, mm)**
2-AIB.HCl salt (70mg/mL)	20	24	22
2-AIB.HOAc salt (70mg/mL)	19	18	20

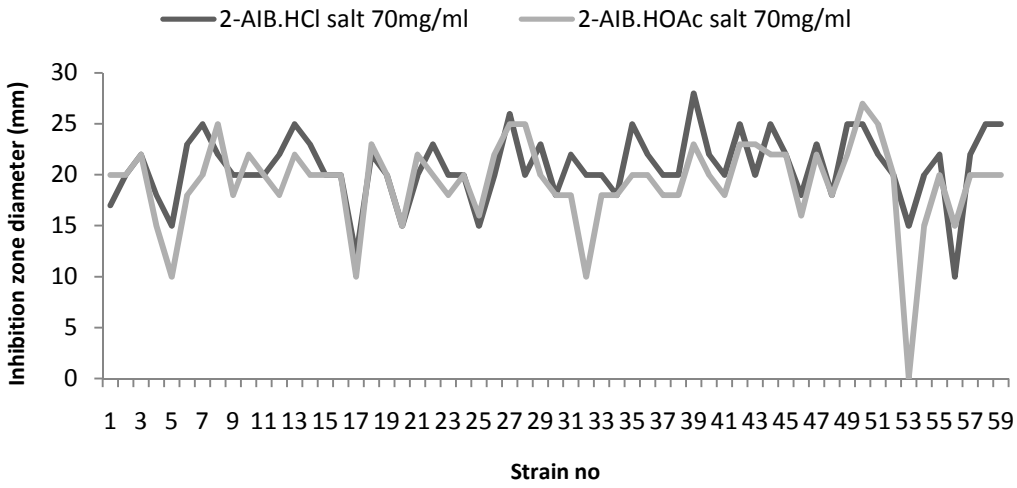


Fig. 2. Sensitivity against ESBL (+) *E. coli* and inhibition zone diameter

Allen et al. researched polyhexamethylene biguanide against *E. coli* treatment and they reported that it is very effective. They described the effect of biguanides based on interaction with bacterial nucleic acid [8]. Furthermore, in a new study Philips et al. demonstrated that polyhexamethylene biguanide used *Pseudomonas aeruginosa* wound model [28]. In other study Banovic et al. found that polyhexamethylene biguanidehas comparable *in vitro* antimicrobial efficacy to chlorhexidine for skin and wound antisepsis. So, we hope that 2-AIB.HCl and 2-AIB.HOAc may also use skin and wound antisepsis [29].

Before reaching any conclusion, we should declare limitation of this study. Limited number of bacteria was tested, It may be better if we could use more and different bacteria. Another limitation that we could not do toxicity test and we couldn't do laboratory animal tests.

Aminoindane and cyanoguanidine that were used for biguanide synthesis are commercially accessible. Moreover, the synthesis of biguanide is a pretty easy single-step approach.

Based on the results that we have, we think that these substances can be of use in hospitals to clean hospital surfaces and hospital floors as disinfectants, they may be used in wound care and skin antiseptics. But, this situation needed *in vivo* tests.

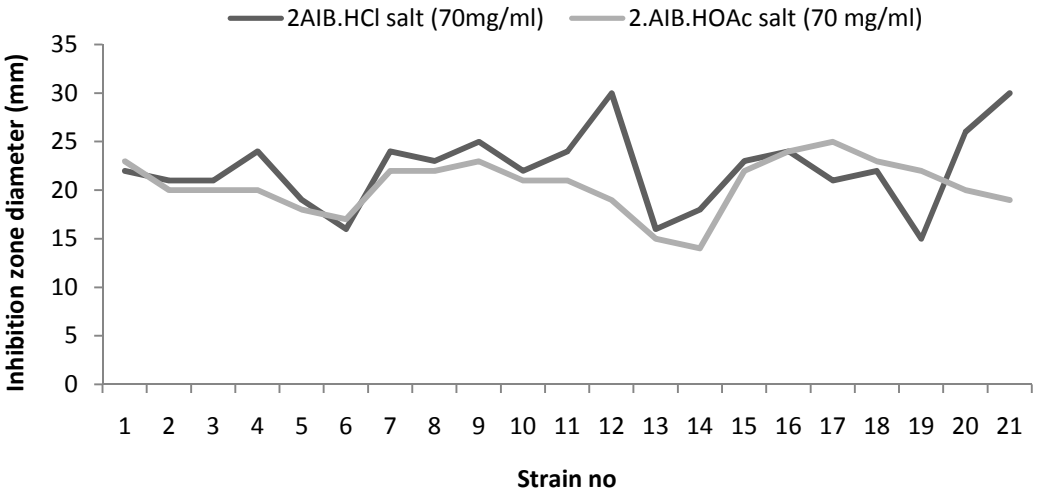


Fig. 3. Sensitivity against *Acinetobacter baumannii* and inhibition zone diameter

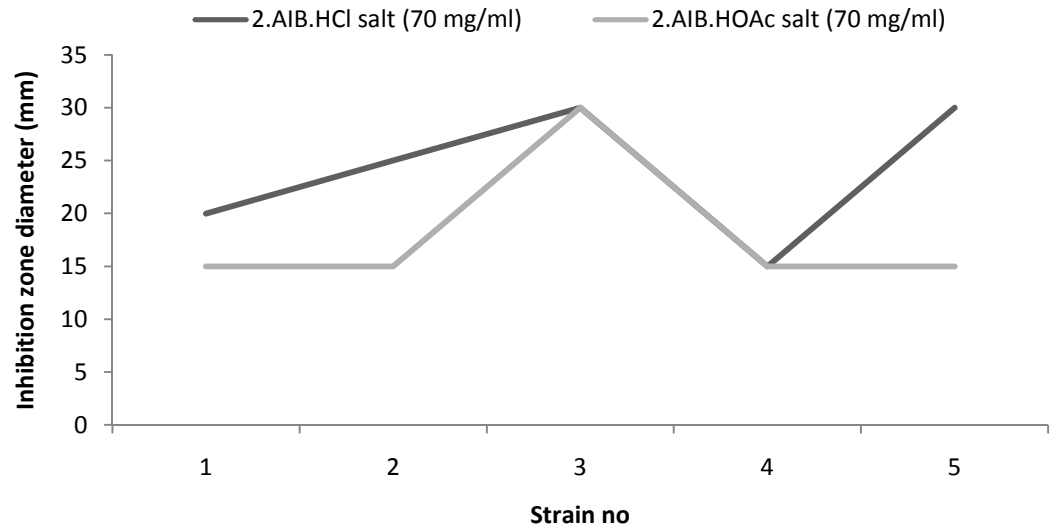


Fig. 4. Sensitivity against *Klebsiella pneumoniae* and inhibition zone diameter

First time with this study 2-AIB salts were found effective against MDR-GNB. Based on our findings, when used in a concentration of the 70 mg/mL, 2-AIB.HCI and 2-AIB.HOAc salts were effective against MDR-GNB.

4. CONCLUSION

Nowadays, antimicrobial resistance is a global issue. Unfortunately most of antibacterial drugs are not effective against to MDR-GNB. If this problem cannot be resolved within the next decade, thousands of people will die in ICUs. These new biguanide derivatives that we synthesized have shown to have antibacterial activities against MDR-GNB. We think that these molecules are important because today very limited alternative drugs are available for the treatment of MDR-GNB infections. 2-AIB salts may be used as new alternatives for it. However, many new *in vivo* and *in vitro* tests are needed before recommending them for clinical use. *In vivo* tests will be made later when we have the ethical requirement to achieve.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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