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Investigation of the Antibacterial Activity of Polyhexamethylene Biguanide (PHMB) Against Multidrug-Resistant and Standard Bacterial Isolates

Polihekzametilen Biguanid'in (PHMB) Çoklu İlaç Dirençli ve Standart Bakteri İzolatlarına Karşı Antibakteriyel Etkinliğinin Araştırılması

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Abstract

Objective: The rise of multidrug-resistant (MDR) bacterial strains in healthcare facilities presents a significant challenge for infection management. Polyhexamethylene biguanide (PHMB), a next-generation antiseptic, offers broad-spectrum antimicrobial activity in addition to low cytotoxicity and environmental safety. This study aimed to evaluate the antibacterial efficacy of PHMB against both standard bacterial strains and clinical isolates, with a focus on its long-term inhibitory effects and concentration-dependent activity.

Material and Methods: Five standard bacterial strains and five clinical isolates were exposed daily to PHMB at concentrations of 0.01%, 0.1%, 0.25%, 0.5%, and 1% over a 14-day period. Colony-forming units (CFU) were quantified following exposure durations of 1, 2, 5, 10, and 30 min, and minimum bactericidal concentrations (MBC) were determined for all strains.

Results: PHMB demonstrated potent antibacterial activity against all tested strains. Concentrations of 0.1% achieved complete growth inhibition throughout the 14-day period. However, at the lowest concentration (0.01%), regrowth was observed from day 10 onward for *Acinetobacter baumannii* and *Enterococcus faecalis*. Significant reductions in CFU counts were noted within the first 5 to 10 min of exposure at all concentrations.

Conclusion: PHMB demonstrated rapid and potent antibacterial activity against both standard bacterial strains and clinical

Öz

Amaç: Sağlık kuruluşlarında çoklu ilaca dirençli (ÇİD) bakteri suşlarının artışı, enfeksiyon yönetimi için önemli bir zorluk oluşturmaktadır. Yeni nesil bir antiseptik olan polihekzametilen biguanid (PHMB), düşük sitotoksosite ve çevresel güvenliğin yanı sıra geniş spektrumlu antimikrobiyal aktivite sunmaktadır. Bu çalışma, PHMB'nin hem standart bakteri suşlarına hem de klinik izolatlara karşı antibakteriyel etkinliğini, özellikle uzun vadeli inhibitör etkileri ve konsantrasyona bağlı aktivitesine odaklanarak değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntemler: Beş standart bakteri suşu ve beş klinik izolat, 14 günlük bir süre boyunca günlük olarak %0,01, %0,1, %0,25, %0,5 ve %1 konsantrasyonlarında PHMB'ye maruz bırakılmıştır. 1, 2, 5, 10 ve 30 dakikalık maruziyet sürelerinin ardından koloni oluşturan birimler (CFU) sayımları yapıldı ve tüm suşlar için minimum bakterisidal konsantrasyonlar (MBC) belirlendi.

Bulgular: PHMB, test edilen tüm suşlara karşı güçlü antibakteriyel aktivite gösterdi. %0,1 ve üzeri konsantrasyonlar, 14 günlük süre boyunca tam büyüme inhibisyonu sağladı. Ancak, en düşük konsantrasyonda (%0,01), *Acinetobacter baumannii* ve *Enterococcus faecalis* için 10. günden itibaren yeniden büyüme gözlemlendi. Tüm konsantrasyonlarda, maruziyetin ilk 5 ila 10 dakikasında CFU sayılarında önemli azalmalar kaydedildi.

Sonuç: PHMB, düşük konsantrasyonlarda bile hem standart bakteri suşlarına hem de klinik izolatlara karşı hızlı ve güçlü antibak-



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isolates, even at low concentrations. Its effectiveness at low concentrations provides significant advantages in terms of reducing cytotoxicity and environmental impact. Further research is warranted to optimise dosing regimens, evaluate the risk of resistance development, and assess the long-term safety of PHMB to fully realise its clinical potential.

Keywords Antiseptic • PHMB, antibacterial activity • multidrug-resistant bacteria • healthcare-associated infections • infection management

INTRODUCTION

The term antiseptics, introduced in the late 18th century, refers to the destruction or inhibition of pathogenic microorganisms on living tissue (1). By the mid-19th century, the integration of antiseptic techniques into surgical practice, pioneered by Joseph Lister, marked a milestone in modern medicine (2). Early chemical disinfectants, such as hydrogen peroxide, demonstrated effective pathogen control, but their clinical use was often limited due to adverse effects, including systemic absorption and tissue toxicity (3).

The advent of antibiotics in the 20th century revolutionised infection management, which consequently led to a diminished focus on antiseptics. However, the emergence and proliferation of antibiotic-resistant nosocomial pathogens have reignited the interest in antiseptics as vital components of infection prevention and control strategies (4). Among the newer antiseptic agents, polyhexamethylene biguanide (PHMB)—a cationic polymer—has garnered attention for its broad-spectrum antimicrobial activity, low cytotoxicity, and environmental safety profile (5, 6). PHMB is widely used in healthcare settings for wound care, surface disinfection, and general infection control (4). Its mechanism of action involves the disruption of microbial membranes and interference with intracellular targets, including DNA, rendering it effective against a wide array of bacteria as well as certain viruses and fungi (7, 8). Despite its demonstrated antimicrobial activity, critical questions remain about how PHMB performs over extended periods and at varying concentrations.

The increasing prevalence of multidrug-resistant bacterial strains in healthcare settings poses a considerable challenge to infection management. While the antibacterial properties of PHMB have been investigated, much of this research has been confined to standard laboratory strains. However, clinical isolates often display unique phenotypic and genotypic characteristics that can significantly impact their susceptibility to antimicrobial agents. Addressing these differences is critical for evaluating PHMB's full potential in combating resistant pathogens (9).

teriyel aktivite gösterdi. Düşük konsantrasyonlardaki etkinliği, sitotoksitesi ve çevresel etkiyi azaltma açısından önemli avantajlar sağlamaktadır. PHMB'nin klinik potansiyelinin tam olarak ortaya çıkarılması için dozaj rejimlerini optimize edilmesi, direnç gelişimi risklerinin belirlenmesi ve uzun vadeli güvenliğini değerlendirmesi amacıyla daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler Antiseptik • PHMB • antibakteriyel aktivite • çoklu ilaca dirençli bakteriler • sağlık hizmeti ilişkili enfeksiyonlar • enfeksiyon yönetimi

In this study, we aimed to evaluate the long-term inhibitory effects of different concentrations of PHMB against both laboratory and clinical bacterial strains. The inclusion of patient-derived isolates will provide a more comprehensive perspective on the potential application of PHMB in clinical settings, particularly in combating healthcare-associated infections.

MATERIAL AND METHODS

Preparation of Polyhexamethylene Biguanide (PHMB) Solutions

PHMB (Guardson Medical Trading Stock Company, İstanbul, Türkiye) was used at an initial concentration of 1%. Dilutions of PHMB (1:2, 1:4, 1:10, and 1:100) were prepared with sterile distilled water at room temperature to achieve final concentrations of 0.5%, 0.25%, 0.1%, and 0.01%, respectively. The undiluted 1% solution was also included in the study.

Selection of Bacteria

The study included five standard bacterial strains obtained from the American Type Culture Collection (ATCC) and five multidrug-resistant (MDR) clinical isolates collected from nosocomial infections at İstinye University Liv Hospital Bahçeşehir in September 2021 (Table 1).

Table 1. Bacterial strains used in the study

Bacterial strains isolated from patients	ATCC bacterial strains
<i>Escherichia coli</i>	<i>Escherichia coli</i> ATCC 25922
<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i> ATCC 27853
<i>Acinetobacter baumannii</i>	<i>Acinetobacter baumannii</i> ATCC 19606
<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i> ATCC 29213
<i>Enterococcus faecalis</i>	<i>Enterococcus faecalis</i> ATCC 29212

Determination of Minimal Bactericidal Concentrations (MBC)

As recommended by standardised protocols for evaluating antimicrobial efficacy (10), all media were freshly prepared on the day of testing. The effect of various concentrations



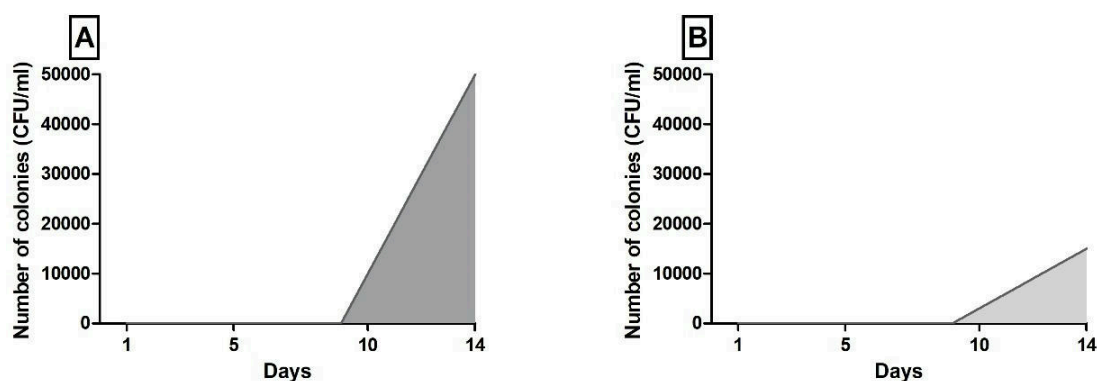


Figure 1. Time-dependent inhibitory effects of PHMB on bacterial strains

A: Growth curve of *Acinetobacter baumannii* ATCC 19606 following exposure to PHMB at 0.01% (1:100 dilution) over 14 days. Complete bacterial inhibition was maintained for the first 9 days, after which regrowth was observed. The curves represent two independent runs (n=2).

B: Growth curve of *Enterococcus faecalis* ATCC 29212 under the same conditions, with bacterial regrowth observed after 9 days.

on day 10. CFU per millilitre were used to quantify bacterial viability, as shown in Figure 1.

Time-Dependent Effect (Long-Term Growth Monitoring)

PHMB exhibited rapid bactericidal activity across all evaluated concentrations and bacterial strains. Significant reductions in CFU counts were observed within the first 5 to 10 min of exposure at all concentrations.

DISCUSSION

PHMB, a member of the polymeric guanidine family, is a next-generation disinfectant. It is widely used in healthcare, food processing, agriculture, and personal care products due to its effectiveness against a wide range of microorganisms, along with favourable properties such as low toxicity, non-corrosiveness, and environmental compatibility (12-15).

In this study, PHMB demonstrated potent antibacterial activity even at low concentrations, effectively inhibiting both Gram-positive and Gram-negative bacteria, MDR strains. Notably, 0.01% PHMB maintained inhibitory effects during the first 9 days against all tested bacteria. However, sustained suppression of *Acinetobacter baumannii* ATCC 19606 and *Enterococcus faecalis* ATCC 29212 beyond day 9 required concentrations of at least 0.1%. The time-dependent decline in efficacy at sub-inhibitory concentrations, along with strain-specific variability, underscores the importance of tailoring dosing regimens to clinical contexts and optimising long-term antimicrobial strategies.

Commercial PHMB formulations generally range from 0.1% to 20% (16), and previous studies have reported effective pathogen inhibition even at low concentrations on various surfaces (17, 18). In line with these findings, our results showed that 0.1% PHMB effectively inhibited all tested bacteria for

up to 14 days. These observations support the potential application of low-dose PHMB, especially in settings where minimising cytotoxicity and environmental impact is critical.

Clinical isolates exposed to continuous selective pressures in healthcare settings often develop adaptive resistance mechanisms, such as upregulated efflux pumps (19, 20) and enhanced biofilm formation (21, 22), which reduce susceptibility to antiseptics and contribute to the persistence of chronic and healthcare-associated infections. This highlights the necessity for ongoing susceptibility testing of clinical isolates to ensure effective infection control.

Each condition was performed in duplicate, which limits the statistical precision. We prioritised breadth across species and multidrug-resistant isolates for this screening-type study; future work will incorporate larger replicate numbers to enable formal hypothesis testing.

CONCLUSION

This study demonstrates that PHMB has strong antibacterial activity against clinical isolates as well as ATCC reference strains in a short time, highlighting its potential as a versatile antiseptic for the management of healthcare-associated infections. Its effectiveness at low concentrations makes it particularly suitable for applications requiring reduced cytotoxicity and environmental burden. However, higher concentrations may be necessary for the sustained suppression of certain pathogens, such as *A. baumannii* and *E. faecalis*. While PHMB's good biocompatibility makes it suitable for infection prevention, further studies are necessary to evaluate its long-term safety and resistance risks. Optimising dosing and investigating combinations with other antimicrobials are key to enhancing its clinical effectiveness and sustaining its antimicrobial action.



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