

Detection of Antibiotic Residues in Powders of Bivalve and Gastropod Mollusc Shells at the Laboratory of the University Clinics of Lubumbashi Using the Disk Diffusion Method

Bora Uzima Akilimali^{1*}, Bidilulinu Mukendi Solomon², Shombo Djoma A³, Lukinga Witanene JP¹, Kasamba Ilunga E⁴ and Kaimbo wa Kaimbo D⁵

¹Department of Specialties, Ophthalmology Service, Faculty of Medicine, University of Lubumbashi, Republic of the Congo.

²Department of Biomedical Sciences, Laboratory Service, Faculty of Medicine, University of Lubumbashi, Republic of the Congo.

³University of Lubumbashi, Faculty of Medicine, Republic of the Congo.

⁴University of Lubumbashi, Department of Biomedical Sciences, university clinics of Lubumbashi.

⁵Faculty of Medicine, Department of Ophthalmology, University of Kinshasa, Democratic Republic of the Congo.

*Correspondence:

Bora Uzima Akilimali, Department of Specialties, Ophthalmology Service, Faculty of Medicine, University of Lubumbashi, Republic of the Congo.

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ABSTRACT

Introduction: Mollusc shells (bivalves and gastropods), known for their ability to bioaccumulate contaminants, may contain antimicrobial substances beneficial to human health. This study explores their potential in combating bacterial infections, particularly ulcerative keratitis.

Materials and Methods: An *in vitro* experiment was conducted at the University Clinics Laboratory of Lubumbashi. Shells of bivalve and gastropod molluscs were ground, extracted in a saline solution, and then tested by disk diffusion against *Escherichia coli* and *Staphylococcus aureus* on Mueller-Hinton agar. The impregnated disks were incubated at 37°C for 24 hours.

Results: Zones of bacterial growth inhibition were observed around the impregnated discs, with an average diameter of 7 mm. These results indicate modest but present antimicrobial activity against both tested strains.

Conclusion: Mollusc shells contain substances active against Gram-positive and Gram-negative bacteria. Although the activity is low, these results justify further research to identify the compounds responsible and evaluate their therapeutic potential, while monitoring the risks associated with antibiotic resistance.

Keywords

Shells (bivalves and gastropods), Ulcerative keratitis, Laboratory of the University Clinics of Lubumbashi.

Introduction

Molluscs, particularly bivalves (mussels, oysters) and gastropods (marine and land snails), play a crucial role in global food security and the economies of coastal and estuarine areas. Their ability to filter water and accumulate substances makes them excellent environmental bioindicators. Among the contaminants of concern are antibiotics, whose intensive use in human and veterinary

medicine, aquaculture, and agriculture has led to their spread into natural environments [1,2]. Even at low concentrations, these compounds can persist and promote bacterial resistance, posing a major public health challenge.

The bioaccumulation of antibiotic residues in shellfish poses a risk to consumers, with potential effects such as allergic reactions, microbial imbalances, or a contribution to antibiotic resistance [3]. Historically, research has focused on soft tissues, but shells, made of calcium carbonate, can also store environmental elements throughout the organism's life [4]. These biomineralized structures

act as environmental archives, revealing environmental conditions and the presence of pollutants [5].

Given the proven detection of substances with antimicrobial activity in the shells of bivalve and gastropod molluscs, and considering their role as bioaccumulators of environmental contaminants, To what extent could these antibiotic residues, once characterized, represent an unexplored source of bioactive molecules capable of offering innovative therapeutic avenues for corneal ulcers (ulcerative keratitis), while simultaneously assessing their potential to exacerbate antibiotic resistance in ophthalmology? It is in this context that the present preliminary study aims to explore the feasibility and relevance of detecting and quantifying antibiotic residues in powdered shells of bivalve and gastropod molluscs.

Methodology

This experiment constitutes an exploratory and qualitative study, of the *in vitro* type, aimed at detecting the presence of substances with antimicrobial activity in unconventional matrices such as mollusc shells. It is part of a preliminary research approach on mollusc shells. The experiment was conducted at the Laboratory of the University Clinics of Lubumbashi, in the Democratic Republic of Congo. The protocol involved a 24-hour bacterial incubation period, preceded by the preparation of shell samples and bacterial inocula. Since the specific bivalve and gastropod mollusc species were not identified, this was a generic approach for this exploratory stage. Laboratory conditions were controlled to ensure the sterility and reproducibility of the disk diffusion tests.

Results

Preparation of Extracts from Bivalve and Gastropod Mollusc Shells

The shells of a **bivalve mollusc** (species not specified) and a **gastropod mollusc** (species not specified) were carefully cleaned to remove any external contamination. They were then mechanically ground separately into fine, homogeneous powders. Each powder was mixed with sterile physiological saline (0.9% NaCl) to solubilize any potentially present water-soluble compounds. The volume of physiological saline used relative to the mass of powder was not specified, but a sufficient ratio was assumed to allow for adequate extraction.



Figure 1: Preparation of bivalve and gastropod mollusc powder using the mixer and vortex.

Bacterial Cultures and Inoculum Preparation

Two reference bacterial strains were used for this test:

- *Escherichia coli* (Gram-negative bacterium)
- *Staphylococcus aureus* (Gram-positive bacterium), given its common use in microbiology.

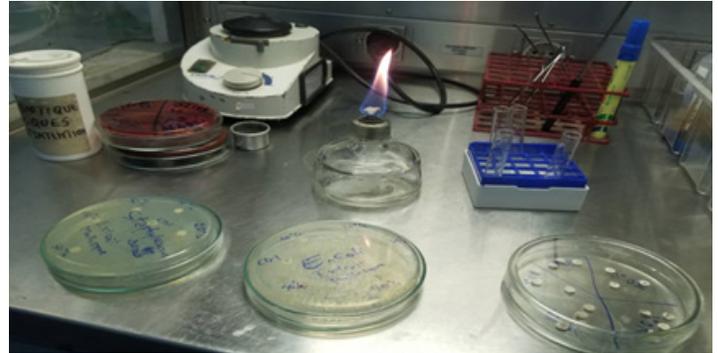


Figure 2: Carpet inoculation of *Escherichia coli* and *Staphylococcus aureus* on Mueller Hinton medium and arrangement of discs impregnated with extracts of shells of bivalve and gastropod molluscs (Laboratory of the University Clinics of Lubumbashi).

Pure colonies of each strain, obtained from fresh cultures, were resuspended in sterile physiological saline. The turbidity of these suspensions was adjusted to a standard equivalent to 0.5 McFarland, which corresponds to a bacterial concentration of approximately $1-2 \times 10^8$ colony-forming units per milliliter (CFU/mL). This adjustment ensures a uniform and reproducible bacterial density on Petri dishes.

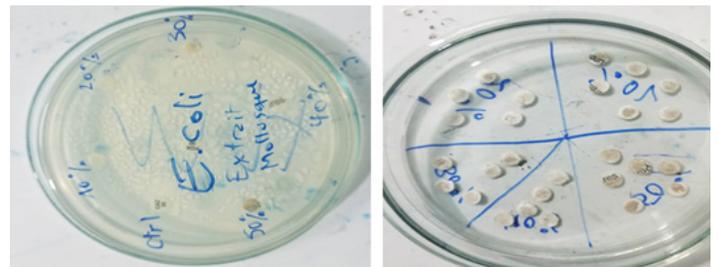


Figure 3: Preparation of antibiograms by diffusion on Mueller Hinton medium with *Escherichia coli* and *Staphylococcus aureus* and discs impregnated with mollusc shell extracts (Lubumbashi).

Inoculation of Petri Dishes

Mueller Hinton Agar (MHA) culture medium was prepared according to the manufacturer's instructions, sterilized, and poured into sterile Petri dishes to obtain a uniform agar thickness. After solidification and partial drying (to avoid excessive surface moisture), each dish was carpet-inoculated with the bacterial inoculum.

- A series of boxes were inoculated with the *Escherichia coli* suspension.
- Another series of plates was inoculated with the *Staphylococcus aureus* suspension.

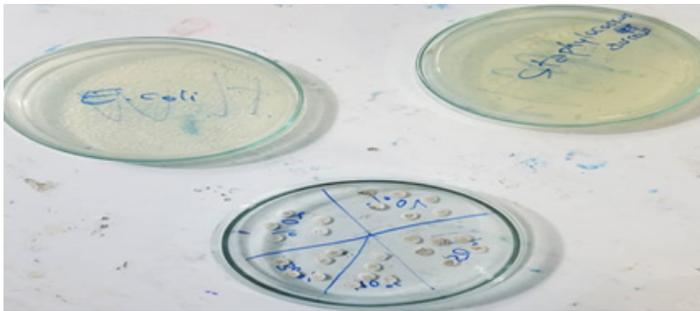


Figure 4: Carpet plating was performed by dipping a sterile swab into the bacterial suspension, squeezing the excess liquid against the tube wall, then rubbing the swab over the entire surface of the agar in three different directions, before swabbing around the edge of the plate. This technique ensures confluent bacterial growth after incubation.

Preparation and Application of Impregnated Discs

Filter paper discs, previously cut using a hole punch, were sterilized. These discs were saturated with the previously prepared solution of powdered **bivalve** or **gastropod shell**. Excess liquid was drained off if necessary.

Five impregnated discs were delicately and equidistantly placed on the surface of the bacterial mat on each Petri dish, using sterile forceps.

- A box containing the *Escherichia coli* mat received 12 discs impregnated with **gastropod shell extract**.
- A box containing the *Staphylococcus aureus* mat received 8 discs impregnated with **gastropod shell extract**.
- A box containing the *Escherichia coli* mat received 15 discs impregnated with **bivalve shell extract**.
- Another box containing the *Staphylococcus aureus* mat received 15 discs impregnated with **bivalve shell extract**.

Incubation

The Petri dishes were incubated at 37°C in an incubator for 24 hours. This temperature and incubation time are optimal for the growth of the bacterial strains used and allow for sufficient diffusion of the antimicrobial substances.

Analysis Results

After 24 hours of incubation, observation of the Petri dishes revealed the confluent growth of bacterial mats in areas unaffected by the discs. **Gastropod shell extract, small areas of bacterial growth inhibition** were observed. The average size of these inhibition zones was measured at approximately 7 mm in diameter. These inhibition halos were visible on both plates inoculated with *Escherichia coli* (a Gram-negative bacterium) and those inoculated with *Staphylococcus aureus* (a Gram-positive bacterium).

Similarly, discs impregnated with **bivalve mollusc shell extract** also showed **faint areas of bacterial growth inhibition**. The size of these inhibition zones was also approximately 7 mm in diameter on average. These inhibition halos were observed on both bacterial strains tested, *Escherichia coli* and *Staphylococcus aureus*.

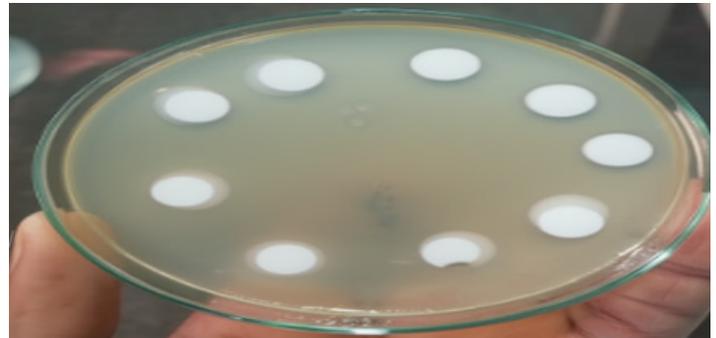


Figure 5: Inhibition of bacterial growth by gastropod shell extract.

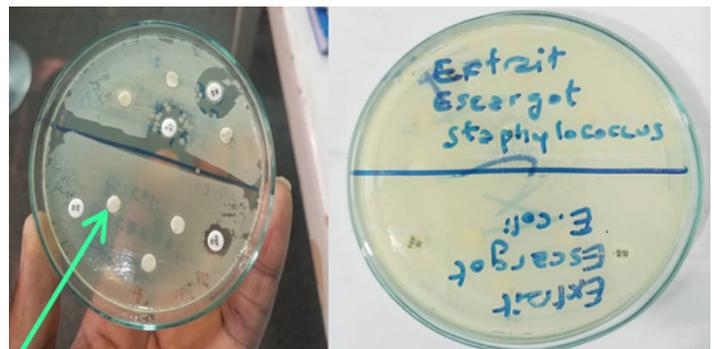


Figure 6: Inhibition of bacterial growth by bivalve mollusc shell extract.

These results suggest that shell extracts from both types of molluscs (gastropods and bivalves) contain substances with weak inhibitory activity against the Gram-negative and Gram-positive bacteria tested.

Discussion

This exploratory study, conducted at the University Clinics Laboratory of Lubumbashi (DRC), aimed to detect the presence of antimicrobial substances in the shells of bivalve and gastropod molluscs. Antibiotic susceptibility testing by disk diffusion revealed zones of inhibition (≈ 7 mm) around disks impregnated with shell extracts, inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* after 24 h of incubation at 37°C.

These results confirm that shells, as biomineralized matrices, can incorporate bioactive compounds from the environment or produced by the molluscs themselves. Indeed, these organisms, lacking an adaptive immune system, mobilize their innate immunity through the production of antimicrobial peptides (AMPs) and other bioactive molecules [6]. Previous work has already highlighted the intrinsic antimicrobial activity of shells [7].

Bioaccumulated antibiotic residues and natural substances raises therapeutic perspectives, particularly in the treatment of ulcerative keratitis, a serious eye condition often resistant to conventional antibiotics [8]. The potential of marine compounds in the development of new antimicrobial agents is well documented [6,7].

However, any clinical application must be preceded by rigorous chemical characterization and pharmacological evaluation of the active molecules. The small size of the inhibition zones suggests modest activity or sub-inhibitory concentrations. Uncontrolled use could promote the selection of resistant strains [2]. Therefore, before any therapeutic use, particularly in ophthalmology, standardized bacteriological susceptibility testing is essential. This preliminary study opens promising avenues for environmental monitoring of antibiotics and the search for new molecules, while highlighting the need for a cautious and methodical scientific approach.

The demonstration of inhibitory activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) in gastropod shell extracts suggests the presence of active compounds with a broad antimicrobial spectrum. This ability to act on bacteria belonging to distinct phylogenetic groups is typical of many antibiotics or natural antimicrobial substances. However, the small size of the observed inhibition zones, approximately 7 mm in diameter, warrants particular attention. This may be explained by a low concentration of the active compounds, an intrinsically moderate activity of the extracted substances, or even by the very nature of these compounds, which could be antimicrobial peptides, secondary metabolites, or other bioactive molecules of natural origin. Furthermore, the inherent limitations of the disk diffusion method, particularly the solubility, stability, and diffusion of the molecules in the agar medium, may also influence the size of the halos.

The hypothesis that gastropod shell powder contains antibiotic residues or antimicrobial substances active against Gram-positive and Gram-negative bacteria therefore appears plausible. Shells, as inert calcareous structures, can serve as bioaccumulation matrices for persistent substances present in the environment. Gastropods, through their filter-feeding lifestyle or diet, are likely to ingest residues from water, sediments, or their food.

To confirm this hypothesis, further investigations are necessary. These would include precisely quantifying potential residues using sensitive analytical techniques such as liquid chromatography coupled with mass spectrometry (LC-MS/MS), identifying the exact nature of the antimicrobial compounds, determining the minimum inhibitory concentration (MIC) of the extracts for the tested strains, and assessing inter-individual and inter-species variability in the accumulation of these substances.

The discovery of inhibition zones, albeit small (≈ 5 mm), around discs impregnated with extracts from bivalve and gastropod shells on reference strains is a significant finding in a preliminary study. It supports the idea that shells can contain antimicrobial substances, whether antibiotic residues [3] or natural bioactive molecules [6]. Until now, research has primarily focused on mollusc soft tissues due to their direct role in food security [9,10]. The growing interest in shells, which can store environmental information over the long term [5], thus opens new avenues for ecological monitoring.

The existence of intrinsic antimicrobial activity in shell extracts also raises the question of their therapeutic potential, particularly in the treatment of ulcerative keratitis. These often serious eye infections are a major cause of blindness, and their management is complicated by the increasing resistance of pathogens to conventional antibiotics [8]. The idea that compounds derived from mollusks could offer new therapeutic avenues is appealing and has already been explored in marine organism research [7].

Nevertheless, any clinical extrapolation must be approached with caution. Therapeutic use based on these extracts requires rigorous chemical characterization of the active molecules and evaluation of their *in vitro* susceptibility against specific pathogens. The absence of such data could lead to ineffective treatments or even exacerbate antibiotic resistance through exposure to sub-inhibitory concentrations or non-targeted substances [2]. Only a standardized antibiogram on strains isolated from the patient would guarantee the efficacy and safety of the treatment.

The results obtained thus justify further research aimed at identifying, isolating, and characterizing the compounds responsible for the observed antimicrobial activity, and at evaluating their spectrum of action, mechanism, and potential toxicity. The goal is to determine whether these substances represent genuine therapeutic alternatives or simply indicators of environmental contamination. Although the antimicrobial potential is evident, its intensity remains low. Previous studies have also highlighted the presence of antibiotic residues in mollusc shells, raising food safety concerns. For example, trimethoprim residues have been detected in mussel shells at levels exceeding regulatory limits, while the presence of microplastics has been associated with increased bioaccumulation of antibiotics in bivalve shells.

Regarding the treatment of corneal ulcers, it is therefore imperative to assess the sensitivity of extracts to the causative pathogens before any therapeutic application. This step is essential to guarantee the effectiveness of the treatment and prevent adverse effects related to uncontrolled use.

Conclusion

This preliminary study, using disk diffusion, revealed the presence of antibacterial substances in the shell powder of gastropods and bivalves. The small inhibition zones observed against *Escherichia coli* and *Staphylococcus aureus* suggest a broad spectrum of activity, although the activity remains modest. These results support the hypothesis that shells may contain antibiotic residues or natural bioactive molecules.

The ability of mollusks to bioaccumulate compounds from their environment reinforces their potential role as bioindicators of antimicrobial contamination. This discovery opens up new avenues for environmental monitoring and the search for novel therapeutic substances. However, the clinical use of these extracts requires a rigorous evaluation of their efficacy and safety. Precise chemical characterization and bacteriological susceptibility testing

are essential before any therapeutic application, particularly in the treatment of corneal ulcers.

The potential presence of antibiotic residues in the shells of gastropods and bivalves underlines the importance of continuous monitoring to prevent risks related to antibiotic resistance and ensure consumer safety.

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