Pharma-molecule Transport Across Cell Membranes – Detection and Quantification Approaches by Electrochemistry and Other Methods

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Introduction

Antibiotic resistance in bacteria is a worldwide clinical issue. According to the World Health Organization, the prevalence of antibiotic resistance in bacteria necessitates that infectious diseases caused by resistant bacteria are becoming more difficult to treat, leading to greater stress on healthcare systems worldwide [1]. Due to its rapid progression, antibiotic resistance is expected to become the leading cause of death by the year 2050 [2].

The membrane transport of pharmacologically relevant molecules is directly implicated in antibiotic resistance. Knowledge of the amounts of uptake, binding and transport kinetics, and permeabilities of pharma-molecules is essential for the development of new treatments for antibiotic-resistant infections. To investigate the relationship between membrane transport and antibiotic resistance, a literature review of papers concerning the quantitative monitoring of membrane transport in bacteria was conducted. The results of the literature review suggest a substantial diversity in methods and quantitative information reported by researchers.

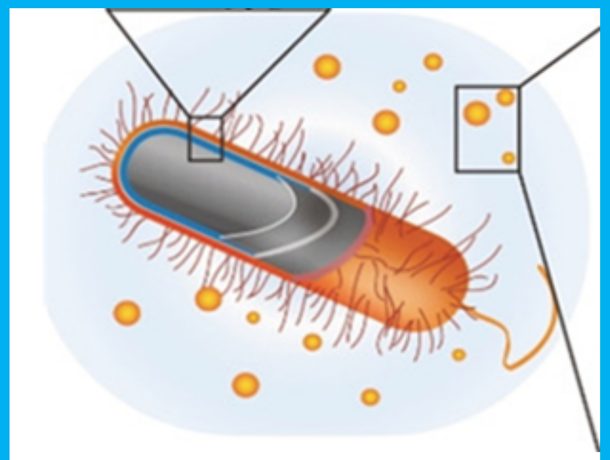

Fig 1. Graphic model of the cell membrane and molecules undergoing membrane transport [3].

Methodology

A comprehensive literature review of papers regarding the quantification of pharma-molecule transport across bacterial membranes was conducted. The key regulators of bacterial transport and the abundance of literature concerned with each pharma-molecule (Fig. 2) were determined. Moreover, each paper was screened for the methods of detection of bacterial transport and relevant quantitative information it reports. Certain papers were compared to find identical numerical parameters detected by different methods.

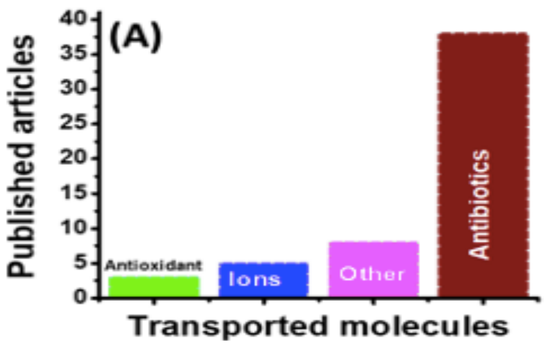

Fig 2. Number of articles published per type of pharma-molecule transported [3].

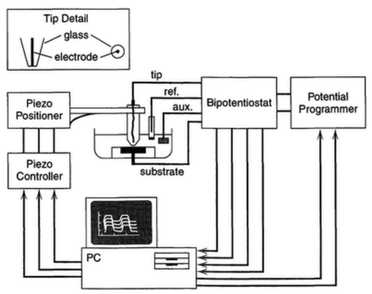

Fig 3. Schematic of a scanning electrochemical microscopy (SECM) setup [4], a method used by Hanness-chlaeger *et al* [5].

Results

1. Bacterial transport is regulated by 3 “key players”: lipid bilayers, porins, and efflux pumps.

2. The literature review yielded a total of 46 papers – 38 regarding antibiotics, 5 regarding ions, 3 regarding antioxidants, 2 regarding metabolites, and 6 regarding unique molecules.

3. The methods of quantification of bacterial transport reported by the review are highly diverse and include electrochemical/electrophysiological (Fig. 3), fluorescence-based, and chromatographic (Fig. 4) methods.

4. The papers surveyed provided a wide range of quantitative information. This includes parameters that are electrochemical/electrophysiological, kinetic, or thermodynamic in nature, as well as concentrations, amounts, or masses of substances permeated by bacteria.

5. Several studies report quantitative parameters for identical pharma-molecules using different methods.

Method	Quantitative information
Scanning electrochemical microscopy [5]	- Average permeability of ascorbic acid = **1.1 ± 0.1 × 10^{-8} cm•s^{-1}** - Average equilibrium constant of ascorbic acid transport = **6.76 × 10^{-2} mM**
Electrophysiology [6]	- Peak current of enrofloxacin transport via wild-type OmpF porin = **-75 pA** - Peak current of kanamycin sulfate transport via wild-type OmpF porin = **-150 pA**
Isothermal titration calorimetry & surface plasmon resonance [7]	- ITC shows pyocin binds to lipopolysaccharide-derived sugars with a K_d of **612 ± 332 nM** - SPR shows pyocin binds to FptA transport protein with a K_d of **6.5 ± 0.4 nM**
Liquid chromatography-tandem mass spectrometry [8]	- Fusidic acid, novobiocin, and erythromycin are low-accumulating, with uptake at **63, 125, and 250 nmol•10^{-12} colony-forming units**, respectively
Single-channel electrophysiology [9]	- Norfloxacin binding to OmpF has a k_{off} of **25000 s^{-1}** at pH 5 and **1561 ± 120 s^{-1}** at pH 7
Ion current fluctuation [10]	- Norfloxacin binding to OmpF has a k_{off} of **9000 s^{-1}** at pH 6
Computer simulation [11]	- Ampicillin transport by mutant E. coli has an outer membrane efflux rate is **0.05 nmol•mg^{-1}•s^{-1}** and the outer membrane influx rate is **0.08 nmol•mg^{-1}•s^{-1}**

Fig 4. Key methods and quantitative information

Conclusion

1. The methods found by the literature review are wide-ranging and diverse.

2. Electrochemical and electrophysiological techniques were frequently utilized, likely owing to their universality, high sensitivity, and high temporal resolution [12].

3. It is important to recognize the advantages and disadvantages of each method of bacterial transport quantification and compare the methods with one another. If progress is to be made in the understanding of antibiotic resistance in bacteria, it is imperative that data from several researchers be considered together.

4. While most papers surveyed reported conventional quantification methods, some papers reported unique, novel methods. In the future, the rapid rise in antibiotics subject to bacterial resistance will necessitate the development of new transport quantification methods.

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Fig 5. Databases used for the literature review.

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