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Research Article

Evaluating Fluoxetine's Growth-Inhibitory Impact on Clinical Isolates of Enteric Bacteria: Harnessing Repurposing Potential

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Abstract

The over-the-counter use and imprudent utilization of antibiotics in recent times have led to the emergence of microbial resistance. This has given rise to an alarming situation, prompting researchers to explore novel strategies for managing infections induced by antibiotic-resistant species. However, the process of drug discovery is time-consuming and expensive, which makes it a challenging endeavor. In recent years, drug repurposing has gained significant attention as an alternative strategy. Notably, several antidepressants have piqued interest as potential candidates for repositioning as antibiotics. Thus, the present study was aimed at investigating the anti-bacterial potential of different classes of antidepressants i.e., fluoxetine, venlafaxine, and phenelzine in clinical isolates of *Escherichia coli, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Shigella dysenteriae,* and *Staphylococcus aureus*. The strains were cultivated on Mueller Hinton agar, with the aforementioned antidepressants or conventional antibiotics. Following a 24-hour incubation period, the extent of growth inhibition was assessed by measuring the zone of inhibition, serving as an indicator of their inhibitory potential. The findings showed that fluoxetine arrested the growth of almost all isolates with maximal effect against *Pseudomonas aeruginosa*. The impact of venlafaxine was exclusively observed in *Enterobacter aerogenes*, with phenelzine demonstrating complete ineffectiveness in bacterial growth suppression. In conclusion, the current study elucidates the efficacy of fluoxetine in clinical isolates, thereby positioning it as a promising contender for the advancement of novel antimicrobial agents. Additionally, our data raises questions regarding the proposed mechanisms of antibacterial action for selective serotonin reuptake inhibitors (SSRIs) and challenges the notion of dysbiosis as a potential mode of action for antidepressants.

Keywords: Fluoxetine, drug repositioning, antimicrobial activity, repurposing potential

1. Introduction

Infectious diseases represent a significant and enduring source of human morbidity and mortality. The extensive diversity within the microbial realm poses a formidable challenge when it comes to devising efficacious therapeutic interventions against the associated maladies. Antibiotics play an indispensable role in the control of infectious diseases; however, their judicious utilization is imperative, as their misuse and overuse have exacerbated the challenge at hand (Liu et al. 2021). Over time, the heavy exploitation of these drugs has resulted in resistance in microbes against existing antibiotic agents. This resistance is now a global issue, and it

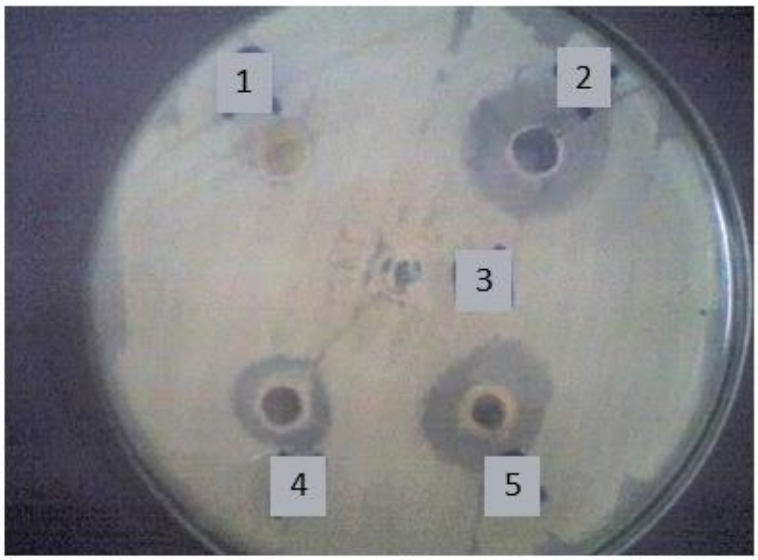

Figure 1: Antimicrobial activity of standard antibiotics and antidepressants against *Salmonella typhi* (1: ofloxacin, 2: fluoxetine, 3: phenelzine, 4: ciprofloxacin, 5: tetracycline).

has resulted in over 700,000 deaths worldwide (Caldara and Marmiroli 2021).

The advent of drug resistance in microbes is explained by two theories (Liu et al. 2021). The first theory believes that antibiotic resistance occurs naturally as it seemed to precede the contemporary selective pressure of clinical antibiotic use (D'Costa et al. 2011). On the other hand, the second theory states that the overuse of antibiotics builds selective pressure, driving the emergence of bacterial resistance (Liu et al. 2021). Although the discovery of new antibiotics could be beneficial for controlling the increase in bacterial resistance, the control of relentless bacterial resistance is impossible to meet with the prolonged process of drug discovery (Yssel, Vanderleyden, and Steenackers 2017). An innovative alternative strategy, attracting global researchers, involves redirecting known and approved drugs to a novel cause (Farha and Brown 2019).

Drug reprofiling or repositioning is a preferable alternative to the modern drug discovery and developmental process (Farha and Brown 2019). It is a system of identifying restorative uses of already existing drugs in the market, cutting off the extensive and arduous process of delivering novel therapeutics in the market (Foletto et al. 2021). There are generally two different principles that have influenced the idea of repurposing drugs. The first principle highlights the ambiguous biological activities of drugs, observed in their side effects. The second principle highlights the shared molecular and genetic pathways among different diseases (Farha and Brown 2019). Using this approach, scientists have started examining the antimicrobial potential of various non-antibiotics to treat infections caused by multi-drug-resistant bacteria (Brown 2015).

Interestingly, a historical analysis revealed that the development of antidepressants is a serendipitous outcome of anti-microbial drug trials conducted in mid last century (López-Muñoz and Alamo 2009). This fortuity laid the foundation of 'the monoamines hypothesis' of depression, which underlies most of the present-day antidepressants (Elhwuegi 2004). It has been evident from several studies that psychological disorders and gut microbiota have a dual-faceted relationship. The disturbance in one can consequently disturb the

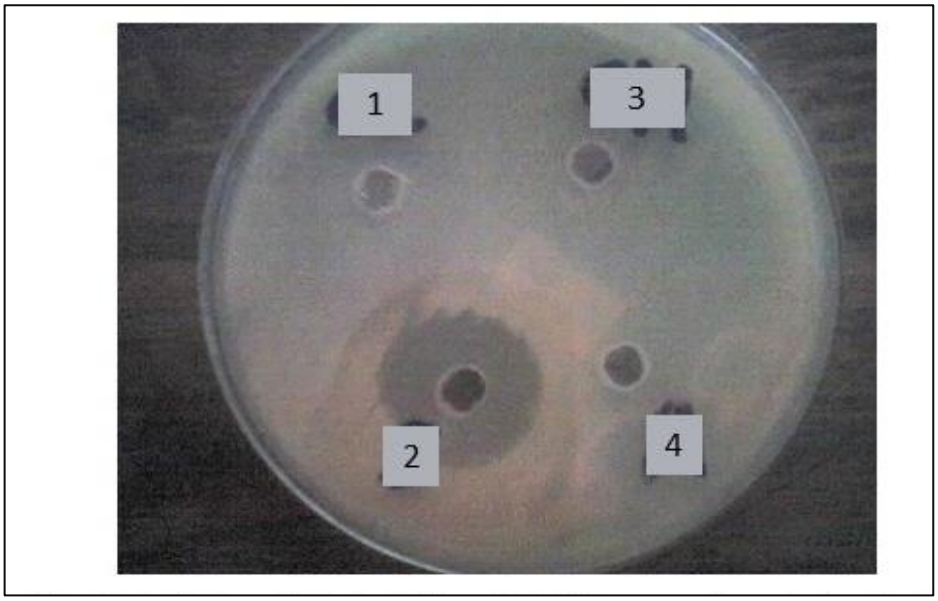

Figure 2: Antimicrobial activity of standard antibiotics and antidepressants against Pseudomonas aeruginosa (1: ofloxacin, 2: fluoxetine, 3: phenelzine, 4: ciprofloxacin).

other (Ait Chait et al. 2020), implicating the role of the "Gut-Brain Axis". Furthermore, the ability of antidepressants to target efflux pumps in microbes and treat infections caused by multi-drug-resistant bacteria has made them a potential candidate (Foletto et al. 2021, Ait Chait et al. 2020), reflecting the possible repositioning potential between antidepressants and anti-microbial agents.

A literature search in this context revealed that several antidepressants such as fluoxetine, sertraline, paroxetine, and escitalopram were reported to possess anti-microbial action (Macedo et al. 2017). Most of these antidepressants belong to the class of selective serotonin reuptake inhibitors (SSRIs), which possibly underlies the assumption that these drugs act by affecting the efflux pumps in microbes (Bohnert et al. 2011, Gjestad et al. 2015). Although the exact mechanism of action is still unknown, many studies have revealed that antidepressants possess promising antimicrobial activities (Ait Chait et al. 2020) and may become a successful candidate for treating bacterial infections in the future. The dearth of reports on the effectiveness of these drugs in disease-causing clinical isolates of microbes and the popular gut-brain axis is pressing the need to study the interaction between antidepressants and enteric bacteria. Keeping this in view, the objective of the present study was to investigate the effectiveness of different classes of antibiotics in clinical isolates of enteric bacteria.

2. Materials & Methods

2.1. Bacterial Strains and Culture Conditions

A total of nine enteric bacterial cultures (*Escherichia coli, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Shigella dysenteriae,* and *Staphylococcus aureus*) were included in this study. The strains of these enteric bacteria were collected from the Reference Culture Collection Laboratory (RCCL) of the Department of Microbiology, University of Karachi. The purity of cultures was checked as per Bergey's Manual of Bacteriology (Holt et al. 1994). The pure cultures

Table 1: *In-vitro* antimicrobial potential of antidepressants and standard antimicrobials against clinical isolates of enteric bacteria.

Enteric Bacteria	Phenelzine (5.8 uM)	Fluoxetine (2.3 uM)	Venlafaxine (2.8 uM)	Amoxycillin/ Clavulanic acid 2:1 (30 ug)	Tetracycline (30 ug)	Ciprofloxacin (5 ug)	Ofloxacin (5 ug)
Escherichia coli	-	27 mm	-	15 mm	10 mm	-	-
Enterobacter aerogenes	-	28 mm	25 mm	22 mm	27 mm	34 mm	32 mm
Staphylococcus aureus	-	27 mm	16 mm	18 mm	30 mm	25 mm	24 mm
Pseudomonas aeruginosa	-	20 mm	-	-	13 mm	-	-
Enterococcus faecalis	-	27 mm	-	20 mm	10 mm	13 mm	-
Salmonella typhi	-	34 mm	-	38 mm	30 mm	28 mm	25 mm
Shigella dysenteriae	-	30 mm	19 mm	28 mm	33 mm	42 mm	35 mm
Proteus mirabilis	-	18 mm	-	17 mm	27 mm	24 mm	23 mm
Klebsiella pneumoniae	-	27 mm	-	14 mm	-	-	-

- No zone of inhibition

were sub-cultured onto Nutrient agar (Oxoid) slants and preserved at 4°C.

2.2. Selection of Anti-depressants and Standard Antibiotics

Anti-depressants: Phenelzine, Fluoxetine, and Venlafaxine were used to determine their anti-enteric bacterial potential. The significance of selecting these drugs may include considerations such as the mechanism of their actions, efficacy, and the specific symptoms or subtypes of depression being targeted. Stock solutions of 10mg/ml were prepared in sterile distilled water. Whereas, standard antibiotics including amoxicillin, ciprofloxacin, and tetracycline were used as a positive control. These antibiotics are used to treat various bacterial infections including urinary, gastrointestinal, and respiratory tract infections.

2.3. Standardization of Inoculum

Each bacterial culture was subjected to a standardization process, wherein its turbidity was adjusted in accordance with the 0.5 McFarland standard. To achieve this, a loopful of culture extracted from the nutrient agar slant was introduced into a sterile normal saline solution, resulting in the suspension of the bacterial culture. Subsequently, the turbidity of this suspension was meticulously compared to that of a reference tube containing the 0.5 McFarland standard.

2.4. Preparation of Antidepressant Stock Solution

From the 10mg/ml stock solutions of the antidepressants, a further five working solutions were prepared by making a two-fold dilution. In this way, working solutions of 4mg/ml, 2mg/ml, 1mg/ml, 0.5mg/ml, and 0.25mg/ml were prepared respectively.

2.5. Screening for Antibacterial Activity

The antibacterial activity of antidepressants was determined using the agar well diffusion assay as described by (Athanassiadis et al. 2009). Overnight culture of enteric bacteria, was swabbed onto the

Table 2: Screening of MIC and MBC of Fluoxetine against *Pseudomonas aeruginosa.*

Fluoxetine Concentration (mg/ml)	*Pseudomonas aeruginosa*		
	Diameter of inhibition zones (mm)	Turbidity in Broth	Bacterial Growth in plates from broth tubes
4mg/ml	39 mm	-	+
2mg/ml	32 mm	-	+
1mg/ml	29 mm	-	+
0.5mg/ml	20 mm	-	+
0.25mg/ml	No zone	+	+
Concentrations between 0.5 mg/ml – 0.25mg/ml			
Fluoxetine Concentration (mg/ml)	**Turbidity in Broth**		
0.45 mg/ml	**-**		
0.4 mg/ml (MIC)	**-**		
0.35 mg/ml	**+**		
0.3 mg/ml	**++**		

Mueller Hinton agar. Additionally, wells of 7mm diameter were punched using a sterile borer, and 80µl of specific concentrations of the drugs were added into the respective wells. One well was dispensed with sterile distilled water that served as a negative control, and the standard antibiotic discs, specific for each bacterial strain, were used as a positive control. All the plates were incubated at 37°C for 24 hours. After incubation, the diameter of the clear zones around each well was measured.

2.6. Determination of Minimum Inhibitory Concentrations (MIC)

To determine the MIC, the broth dilution method was used. Five tubes containing 2ml nutrient broth were inoculated with 200µl of bacterial cultures. Following this, 1 ml of serially diluted concentrations ranging from 0.45mg/ml – 0.3mg/ml of the antidepressants was dispensed in the tubes. One tube was designated as the positive control, devoid of any antidepressant concentration, while another tube solely contained nutrient broth to function as the negative control. Following a 24-hour incubation period at 37°C, the turbidity of each working tube was compared to that of the negative control, and the outcomes were recorded.

2.7. Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC, all the concentrations of the drug, that inhibited bacterial growth in the previous step of MIC assessment, were selected. A volume of 100µl from the culture, drug, and broth suspension was evenly spread on Mueller Hinton agar plates. One plate was inoculated with 100µl of bacterial culture for the positive control, while another remained uninoculated as the negative control. After 24-hour incubation at 37°C, the results were observed.

3. Results & Discussion

Addressing the drug development challenges can be eased through drug repositioning or repurposing. The discussion on the antimicrobial capabilities of antidepressants has generated a debate, with existing literature referring to their effects mainly on commercially available cultures. These cultures may vary from the disease-causing clinical isolates, studied in our research. Our obtained data indicated that fluoxetine successfully impeded the growth of nearly all nine isolates, underscoring its broad-spectrum potential in clinical environments as depicted in

Table 1, Figures 1 and 2. Moreover, there are previous studies, such as the work by (McGovern, Hamlin, and Winter 2019), that evaluated the antimicrobial activity of some SSRIs against *E. coli* and *S. aureus*. Among nine isolates, *P. aeruginosa* was found to be more sensitive against fluoxetine (20 mm zone of inhibition) as compared to standard antibiotics. This data is in line with existing literature, which demonstrated the effectiveness of fluoxetine against various forms of microorganisms such as bacteria (Munoz-Bellido, Munoz-Criado, and Garcıa-Rodrıguez 2000), viruses (Ulferts et al. 2013), fungi (Lass-Flörl et al. 2001), and algae (Brooks et al. 2003). Despite several such reports, not much is known about its mechanism of antibacterial action.

Furthermore, the literature also revealed that most of the antidepressants acting as antimicrobials belong to the class of SSRIs. This possibility underlies the hypothesis that these drugs may interfere with the efflux system of microbes (Bohnert et al. 2011, Gjestad et al. 2015). Nevertheless, our findings indicate that this presumption might not be true, as another medication within the serotonin-norepinephrine reuptake inhibitors (SNRI) class, demonstrated effectiveness in inhibiting the growth of *Enterobacter aerogenes*. In contrast, phenelzine was reported to be ineffective as an antibacterial (Ayaz et al. 2015). Hence, it can be deduced that antimicrobial action is not the general potential of antidepressants and may not be associated with bacterial efflux system; and further work is required to delineate the mechanism of action of SSRIs.

Moreover, we assessed the MIC of the most effective drug fluoxetine against *P. aeruginosa*, the most susceptible strain, in our investigation, which was determined to be 0.4mg/ml, as shown in Table 2. Despite its inhibitory effect at a minimum concentration of 0.4mg/ml, any other higher concentration of fluoxetine showed no promising bactericidal effect *(*Table 2, *Figure 2)*. This indicates that repurposing antidepressants as antimicrobials might not be very efficient in treating resistant infections, as they can only inhibit bacterial growth for some time, but they are not capable of completely killing the bacterial cells.

These days, the popular gut-brain axis is also pressing the need to observe the interaction between antidepressants and microbes from a much broader perspective than drug repositioning alone (Macedo et al. 2017). Considerable debate surrounds the potential role of gut-microbial dysbiosis as a mode of action for antidepressants. Additionally, previous data indicates that the impact on the gut microbiome could be one of the mechanisms underlying the antidepressant effects (O'Mahony et al. 2015). However, our data revealed that among various classes of antidepressants, only SSRIs have the ability to interfere with the growth of enteric microbes. Hence, the aforementioned nexus between antidepressant action and enteric bacteria may not be true. Moreover, the literature also does not reveal a single report that could prove such an association.

4. Conclusions

In conclusion, among various classes of antidepressants, only fluoxetine (SSRI) significantly arrests the growth of bacteria and justifies repurposing it as an antimicrobial agent. Our data also implicates doubt over both the suggested mode of action of SSRIs and the role of gut dysbiosis as a possible mode of action of antidepressants.

Conflict of Interest

The authors declare no conflict of interest.

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Study Approval

This study was approved by the Department of Microbiology, University of Karachi, Pakistan.

Consent Forms

NA.

Authors Contribution

AS, JS, AS, and YR carried out all the data collection, bench work, and manuscript writing. GA and RS helped in data collection and statistical analysis. YR conceptualized and supervised the study.

Data Availability

Data is available upon reasonable request from the corresponding author.

Acknowledgment

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