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Direct Analysis of Substandard Antibiotics Sensitivity Pattern against Common Isolates of Pathogens *via* Modified Oucherlony Technique: A *in vitro* Study in Karachi

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Research Article

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ABSTRACT

Introduction: Medicines are blessing for mankind, which use for treating, curing the disease and deliver relieve in pain and manage the illness sign and symptoms. There are varieties of substitute present in the market of same generic medicines. Some are cost-effective, several are substandard in quality; some are inefficient in efficacy and ultimately are not yielding same effect against the claim. The purpose of the study is to explore the differences in the quality of locally manufactured antibiotics in Pakistan by using modified Oucherlony technique.

Design and participants: In order to conduct this Pilot study, 3 samples of antibiotics from interior and frontier areas of Pakistan were selected as candidate medicines.

Measurements: Data source is primary and samples are randomly collected addressing the research objective. Modified Oucherlony double immunodiffusion technique is used to assess antibacterial and bacteria resistance susceptibility pattern of 3 brands of locally manufactured cheap price medicines against the commonly isolates UTI pathogens.

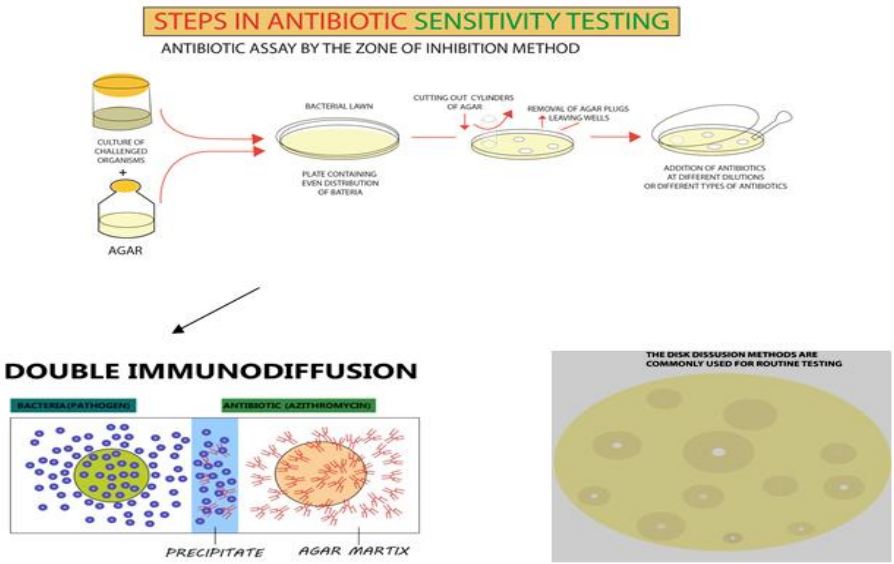
Findings and conclusion: This study shows that out of three brands of antibiotics, two of the brands of azithromycin are highly resistant against the UTI pathogens; however one of the brands are resistant to *S. aureus*, *Acinetobacter*, *E. coli* but little activity showed in the enterococcus bacteria.

INTRODUCTION

Medicines are used to take care of the patient by treating, preventing the disease and provide relieve in pain and managed the sign of the ailment. In order to get a desired outcome, medicines should be safe and effective for human health. It gives the trust and conviction to the patient whom prescribed. This can be change into mistrust when medicine produced entirely different action from its own action and this is due to substandard medicine or counterfeit medicine.

Multiple antibiotic resistances in bacterial populations are a pervasive and growing clinical problem, which is recognized as a threat to public health [1]. Urinary Tract Infections (UTIs) are one of the most common bacterial infections in humans, both in the community as well as in the hospital settings [2-4]. Urinary tract infection is a widespread clinical episode that influence patients within all ages, sexes, related with diversity of clinical setting and it is graded as second to respiratory tract infection, in cases seen in developing countries [5-8]. There are number of treatments and medications present in the market for complicated to uncomplicated UTI which are successfully recover the patient (Figure 1) [9-10].

Figure 1. Steps in antibiotic sensitivity testing P.



But the infection is commonly present and people who are living in the rural areas need highly cost-effective treatment with medications. So, for the fulfillment of the need of the patients, different brands are present in the market which is cost effective. Due to cost effective therapy, different drugs suffered in quality issues. This may lead to the issue of counterfeit, substandard quality medicines or fake medicines trading.

There is growing universal concern regarding counterfeit medications. In particular, counterfeit antimicrobial drugs are a threat to public health with many devastating consequences for patients, increased mortality and morbidity and emergence of drug resistance. In addition, physicians treating these patients lose their confidence in the medications used and report high levels of bacterial resistance.

Several methods have been used for the detection of substandard medications including inspection, dissolution assays, colorimetric methods and chromatography techniques such as HPLC, TLC, Mini-lab and mass spectrometry. New technologies such as near infrared spectroscopy and X-ray powder diffraction method have been increasingly used for the detection of counterfeit antimicrobials.

Counterfeit word in medicine, is quite mystifying to define and it's quite controversial and unclear and because of a blunder, there is no need to classify. Medicines may be a medicine having erroneous ingredient or may be inadequate active ingredient or adequate active ingredient or may be active ingredient which is different from the mentioned in the label, or has incomplete information in the label, or drug is expired or has no expiry is mentioned, or medicine having fake packaging or may contain strength other than the mentioned in the label. As far as types of the counterfeit drug are concerned along with their extent and degree, it can be categorized into six classes (WHO, official website)

- Products devoid of active ingredients i.e. about 32.1%
- Products with erroneous number of active ingredients, 20.2%
- Products with incorrect ingredients, 21.4%,
- Products with accurate magnitude of active ingredients but with forged covering, 15.6%
- Duplicate of an original product, 1%; and
- Products with elevated point of adulteration and contaminants, 8.5%.

Some studies have been done in the past to differentiate between the counterfeit and substandard medicine. While some researcher argued that it is still in questioned mark that there is no such difference between the substandard drug and counterfeit drugs. Some researcher studied that no accurate and approved active ingredient is present inside the medicine. On the other hand, some argued that counterfeit is due to insufficient active ingredient in the drug instead of enough.

Fake medicine marketing, selling and buying is one of biggest challenge to whole world. This threat is not only for under developed countries but also for developed country. Forged is most often allied with the simulated of major brand-name consumer goods.

Many studies have been conducted in past related to producing, selling and marketing of counterfeit medicine. Some of the work has also been done in Pakistan but the reasons of selling purchasing and marketing couldn't understand. In particular areas in Africa, Asia, and Latin America, chances of purchasing a counterfeit drug may be higher than 30%. The majority of information on the epidemiology of counterfeit drugs is kept undisclosed by the pharmaceutical industry and by governmental organizations. Drug companies take up investigators to capture and make possible the shutting down of counterfeit industries, but this happens immeasurably secretive.

In this study at Memon Medical Institute Hospital Pakistan, have tested directly on the bacterial pathogen's method. The aim of our study is to check the susceptibility or resistant pattern in the isolated from various clinical specimens (*Staph aureus*, *Acinetobacter, E. coli, and Enterococcus*) in microbiological laboratory and compare it with suspected substandard antibiotics purchase from interior area of Sindh and frontier area of Pakistan. Due to no funds and support, we prefer to do pilot study.

METHODOLOGY

Antibacterial sensitivity and resistance of different drugs were tested by standard Kirby Bauer zone diameter method with Oucherlony double immune diffusion technique (a combination of Kirby Bauer method, combined with Minimum inhibition concentration technique and Oucherlony technique of well-diffusion method). This technique is mostly used in the detection, identification and quantification of antibodies and antigens. In total, we used three different drugs to find out the above activity against the *E. coli*, Klebsiella, Pseudomonas, *Staphylococcus aureus*. Additionally, antibacterial activity was determined by Agar well-diffusion method.

Procedure

One hundred micro liters (100 µl) of standardized inoculum (0.5 Mac-Farland) of each test bacterium including (*E. coli*, Klebsiella, Pseudomonas, *Staphylococcus aureus*) were inoculated on molten Mueller-Hinton agar, subsequently homogenized and poured into sterile plates. Standard corn borer of diameter (16 mm) was used to make uniform wells into which aqueous solution of test antibiotic of strength 8, 16, 32, 64, 128 ug/ml was added (250 µl). The test was done in triplicates to find out the exact results. Standard antibiotic as control was used as positive control. Sodium phosphate buffer (50 mM) alone was used as a negative control. The plates were then incubated at 37°C ± 1°C for 24 hours and the zone of inhibition was measured with the help of standard scale.

RESULTS

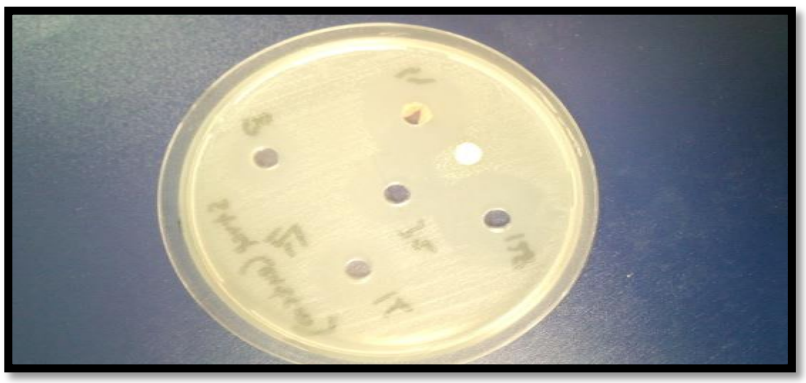
Following are results of three antibiotics Ery-Pack, Zith, Azomin

One of the locally manufactured antibiotics has fully resistant on *Acinetobacter, Staphylococcus, and Staph aureus, E. coli, Klebsela and Enterococcus* in all its dilutions. However, the medicines is effective against the streptococcus with the zone of 1.5 mm, 1.7 mm, 1.8 mm, 2.4 mm and 2.5 mm in concentration of 8 µl, 16 µl, 32 µl, 64 ul and 128 µl respectively (Table 1) (Figure 2).

Table 1. Effects of medicines against the Streptococcus with different zones.

Medicine ZITH (Azithromycin)					
Bacteria	Concentrations				
	8 µL	16 µL	32 µL	64 µL	128 µL
Enterococcus	1.5 mm	1.7 mm	1.8 mm	2.4 mm	2.5 mm
Acinitobacter	R	R	R	R	R
S.aureus	R	R	R	R	R
E.coli	R	R	R	R	R
Klebsela	R	R	R	R	R

Figure 2. Zith drug, mueller-hinton agar method.



In Table 2 the second locally manufactured antibiotic Ery-Pack the medicine is fully susceptible against the streptococcus bacteria Figure 2 but show partial resistant against *Acinetobacter* in the concentration of 8 µl and 16 µl while effective in the concentration of 32 µl,64 µl and 128 µl having zone of 1.5 mm, 1.8 mm and 2 mm (Tables 2 and 3) (Figure 3 and 4).

Table 2. Sensitivity pattern of medicine "ery-pack" on pathogens.

Medicine ery-pack (Azithromycin)					
Bacteria	Concentrations				
	8 µL	16 µL	32 µL	64 µL	128 µL
Acinitobacter	R	R	1.5 mm	1.8 mm	2 mm
S.aureus	R	R	R	R	R
E.coli	R	R	R	R	R
Klebsela	R	R	R	R	R
Enterococcus	2 mm	2.5 mm	2.7 mm	3.5 mm	3.6 mm

Figure 3. A. ery-pack drug, B and C. Blood culture sensitivity patterns mueller-hinton Ag.

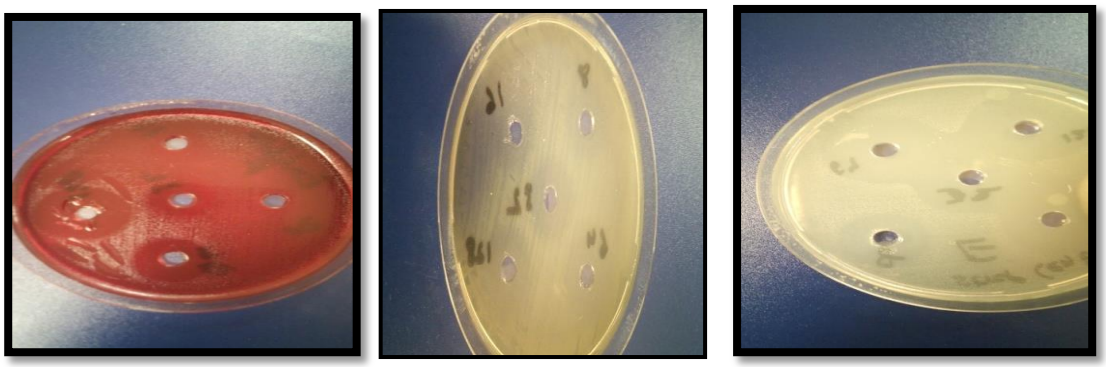
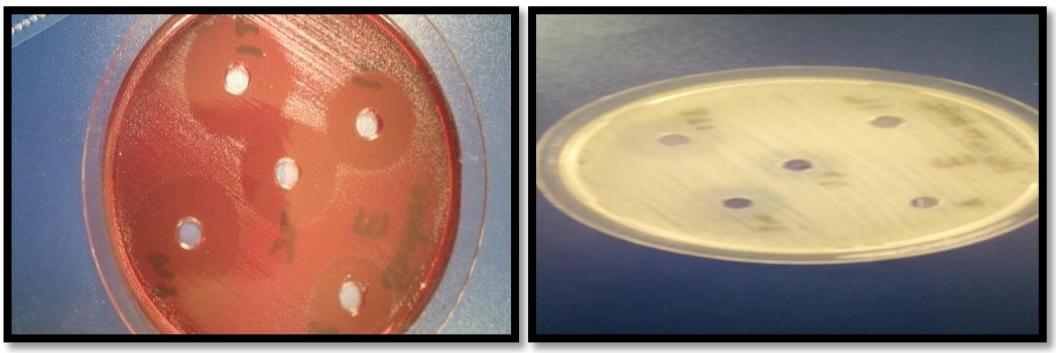


Table 3. Sensitivity pattern of medicine "Azomin" on pathogens.

Medicine Azomin (Azithromycin)					
Bacteria	Concentrations				
	8 µL	16 µL	32 µL	64 µL	128 µL
Acinitobacter	R	R	R	R	R
S.aureus	R	R	R	R	R
E.coli	R	R	1.4 mm	1.5 mm	2.5 mm
Klebsela	R	R	R	R	R
Enterococcus	R	R	1.5 mm	1.8 mm	2.7 mm

Figure 4. Azomin drug A. Blood culture sensitivity patterns, B. Mueller-hinton agar patterns.



DISCUSSION

In this pilot study of an *in vitro* study of different locally manufactured antibiotics in Pakistan, their sensitivity pattern against common isolates UTI pathogens *via* modified Oucherlony technique shows that the locally manufacturing antibiotics are not effective and highly resistance. The sensitivity pattern with standard antibiotics shows high degree of resistance. On the other side, there was 100% resistance with exception of Ery-pack and Zith against enterococci but the zone size did not increase in concentrations indicating that it is not effective similarly antibiotics Azomin against E. *coli* showed sensitivity with no zone increase with concentration leading to suspicion that its efficacy is questionable. It is concluded that there is varying degree of resistance shown by bacteria tested towards standard antibiotics with sensitivity data these antibiotics can be prescribed however on the other hand substandard antibiotics did not show any indicator that they may be prescribed. Comparing with the original antibiotics the antibacterial zone of the locally manufactured antibiotics is very small in size and in some concentration; the medicines didn't produce any effect. This shows that the antibiotics are in inferior quality. However previous studies show that both of the generic of antibiotics is highly sensitive to the Klebsela, *E. coli*, *S. aureus*. There are different reasons which are cause of resistance in bacteria such as clinical conditions, geographic regions, irrational or excessive use of antibiotics. But this is also because of substandard quality of medicines, low cost of inferior or counterfeit antibiotics. Due to poor quality of drugs ingredient or counterfeit medicines trading, this is also a dilemma that we people have to bear resistance in antibiotics. One of the resistances against the bacteria is counterfeit medicines problems in developing countries. Other factors which might be effect on the anti-microbial susceptibility patterns bacterial isolates in much of the developing world is unknown, and little guides pragmatic set down. Susceptibility testing cannot be done willingly because apparatus, workforce, and consumables are inadequate and expensive.

The results can give out undeviating any national effort intended toward reducing the antimicrobial resistance problems of local hospitals. The reasons for the disparity in antimicrobial drug–resistant patterns might be connected to infection control practices or to timing of the introduction of resistant organisms.

CONCLUSION

However, more research is needed to clarify these differences. We believe that our findings represent the prevalent drug resistant situation in different hospitals in Karachi if we have enough funds to test the procedure to majority of the locally manufactured antibiotics supplied in different regions of Pakistan. After analyzing multiple times, our Pilot study shows that out of three brands of antibiotics, 2 of the brands are highly resistant against the UTI pathogens which are azithromycin however one of the brands are resistant to *S. aureus*, *Acinetobacter*, *E. coli* but little activity show in the enterococcus bacteria.

LIMITATIONS

In this study, there are many limitations, but the study indicates that substandard antibiotics are ineffective although they flourish in province of Sindh and frontier side of Pakistan.

DECLARATIONS

Conflict of interest

There is no conflict of interest.

Acknowledgement

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