Direct Analysis of substandard Antibiotics Sensitivity pattern against common isolates of pathogens via modified Outerlony technique-A vitro study in Karachi

Authors:
Hina Sharif*, Sana Sharif², Haroon Diwan³,
¹Mariestopes Society, Pakistan
²University of Saskatchewan, Canada
³Memon Medical Institute Hospital, Pakistan.

*Corresponding Author:
Hina Sharif*,
*Mariestopes Society, Pakistan
E-mail: hina.sharif@mariestopes.org.pk

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 Outerlony 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 Outerlony 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 Conclusions: 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.

Keywords: Inefficacy, resistant antibiotics, substandard quality medicines, cost effective medication. Modified Outerlony technique
Medication: Modified Outerlony technique

![Diagram of STEPS IN ANTIBIOTIC SENSITIVITY TESTING](image)

**Figure 1: Steps involve in Antibiotic Sensitivity Testing**

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.

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 Its Quite Controversial And Unclear And Because Of A Blunder, There Is No Need To Classify [12]. 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 [13] Or May Contain Strength Other Than The Mentioned In The Label [14]. 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%. [15]


Many Studies Have Been Conducted In Past Related To Producing [21], Selling And Marketing [22] Of Counterfeit Medicine. Some Of The Work Has Also Been Done In Pakistan [23] 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%” [24]. 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 [25].

In This Study At Memon Medical Institute Hospital Pakistan, Have Tested Directly On The Bacterial Pathogens 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 (Ery-Pack, Zith, Azomin) 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 Outerlony Double Immune Diffusion Technique (A Combination Of Kirby Bauer Method (1), Combined With Minimum Inhibition Concentration Technique And Outerlony Technique Of Well-Diffusion Method). This Technique Is Mostly Used In The Detection, Identification And Quantification Of Antibodies And Antigens [26]. 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 [27].

**Procedure:** One Hundred Micro Liters (100 Ml) 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 Cork Borer Of Diameter (16 Mm) Were Used To Make Uniform Wells Into Which Aqueous Solution Of Test Antibiotic Of Strength 8, 16, 32, 64, 128 Ug/ml Was Added (250 Ml). 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 ± 1°C For 24 Hours And The Zone Of Inhibition Was Measured With The Help Of Standard Scale.

Ethical Approval Is Obtained By Research Ethics Board (REB) Committee Of Memon Medical Institute.

Results:
Following Are Results Of Three Antibiotics “Ery-Pack”, “Zith”, And “Azomin”:

One Of The Locally Manufactured Antibiotics Has Fully Resistant On Acinetobacter, Staphylococcus, And Staph Aureus, E. Coli, Klebsiella And Enterococcus In All Its Dilutions. However, The Medicines Are Effective See (Table: 1) Against The Streptococcus With The Zone Of 1.5mm, 1.7mm, 1.8mm, 2.4mm And 2.5mm (Figure:1) In Concentration Of 8µl, 16µl, 32µl, 64ul And 128µl Respectively.

Table 1: Sensitivity Pattern of Medicine “Zith” On Pathogens

<table>
<thead>
<tr>
<th>MEDICINE Zith</th>
<th>(Azithromycin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACTERIA</td>
<td>CONCENTRATIONS</td>
</tr>
<tr>
<td></td>
<td>8µL</td>
</tr>
<tr>
<td>ENTEROCOCCUS</td>
<td>1.5mm</td>
</tr>
<tr>
<td>ACINITOBACTER</td>
<td>R</td>
</tr>
<tr>
<td>S.AUREUS</td>
<td>R</td>
</tr>
<tr>
<td>E.COLI</td>
<td>R</td>
</tr>
<tr>
<td>KLEBSELA</td>
<td>R</td>
</tr>
</tbody>
</table>

Figure 2: Zith Drug: Mueller-Hinton Agar

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 8µl And 16µl While Effective In The Concentration Of 32µl,64µl And 128µl Having Zone Of 1.5mm, 1.8mm And 2mm Respectively. The Zone Size of the Susceptible Antibiotic
Table 2: Sensitivity Pattern of Medicine “Ery-Pack” On Pathogens

<table>
<thead>
<tr>
<th>Medicine Ery-Pack (Azithromycin)</th>
<th>8µL</th>
<th>16µL</th>
<th>32µL</th>
<th>64µL</th>
<th>128µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACINITOBACTER</td>
<td>R</td>
<td>R</td>
<td>1.5mm</td>
<td>1.8mm</td>
<td>2mm</td>
</tr>
<tr>
<td>S.AUREUS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E.COLI</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>KLEBSELA</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>ENTEROCOCCUS</td>
<td>2mm</td>
<td>2.5mm</td>
<td>2.7mm</td>
<td>3.5mm</td>
<td>3.6mm</td>
</tr>
</tbody>
</table>

Figure 3: Ery-Pack Drug (A): Blood Culture Sensitivity Patterns (B, C): Mueller-Hinton Ag

In The Testing Of Third Antibiotic, It Is Observed The Third Antibiotic Azithromycin Is Partially Effective In The Concentration Of 64ul And 128ul And The Zone Size Of 1mm And 2.5mm Respectively. This Is Similar Against The E. Coli And Enterococcus And Effective In The Concentration Of 32ul, 64ul And 128ul With Zone Size Of 1.4mm, 1.5mm And 2.5mm Respectively In Case Of Enterococcus Bacteria. R Is The Resistance Against The Bacteria.

Table 3: Sensitivity Pattern of Medicine “Azomin” On Pathogens

<table>
<thead>
<tr>
<th>MEDICINE Azomin (Azithromycin)</th>
<th>8µL</th>
<th>16µL</th>
<th>32µL</th>
<th>64µL</th>
<th>128µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACINITOBACTER</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>S.AUREUS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E.COLI</td>
<td>R</td>
<td>R</td>
<td>1.4mm</td>
<td>1.5mm</td>
<td>2.5mm</td>
</tr>
<tr>
<td>KLEBSELA</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>ENTEROCOCCUS</td>
<td>R</td>
<td>R</td>
<td>1.5mm</td>
<td>1.8mm</td>
<td>2.7mm</td>
</tr>
</tbody>
</table>
Conclusions:

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 shows in the Enterococcus bacteria.

Discussion:

In this pilot study of in vitro study of different locally manufactured antibiotics in Pakistan, their sensitivity pattern against common isolates UTI pathogens via modified outlerony technique shows that the locally manufacturing antibiotics are not effective and highly resistant. 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 shown 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 [28, 29]. However, previous studies show that both of the generic of antibiotics are highly sensitive to the Klebsiella, E. Coli, S. Aureus [30].

There are different reasons which are cause of resistance in bacteria such as clinical conditions, geographic regions, irrational or excessive use [30] of antibiotics. But this is also because of substandard quality of medicines [31], low cost of inferior or counterfeit antibiotics [32]. 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 [33, 34, 35, and 36].

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 [37, 38]. 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 [39].

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. 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.

Limitation of the Study: There Are Few Limitation of This Study: (A) This Study Based On Single Generic of Sub-Standard Medicines (Azithromycin). (B) Due To Un-Availability Of The Funds, The Pilot Study Performed On Small Scale.

Future Recommendation Related To This Research: This Study Was Conducted In Small Scale; It Is Recommended For Future To Organize This Study On Larger Scale With Two Or Three Generic Of Sub-Standard Medicine Which Would Give More Clear View Of This Research.

Conflict Of Interest: There Is No Conflict Of Interest.

Acknowledgement: Authors Would Like To Thank To Mr. Sohail Habib, Dr. Rizwan Azami, Dr. Hanif Baig, Ms Nasreen Sultana, Mr. Ghulam Haider Khan, And Ms Zaitoon For Their Moral Support, Help And Encouragement For Study Execution.

Disclaimer: This Is A Pilot Study Due To Unavailability Of Funds.

Author Contribution: Hina Sharif Involved In Study Design, Sample Of Medicine And Manuscript Writing. Sana Sharif Involved In Supporting Literature Search, Microbial Lab Testing And Lab Evidence Based Interpretations. Dr. Haroon Contributed In Revising It Critically For Substantial Intellectual Content And In Research Approved Suggestions. All Authors Read and Approved The Final Manuscript.

Reference:

[15] WHO, Official Website


[20] The Partnership for Safe Medicines Uses the Term Counterfeit Broadly, the Way This Report Uses Falsified


[28] Yoshida Et Al. BMC Pharmacology and Toxicology 2014, 15:13


[34] Esezobo E, Offiong E. In Vitro Studies On Some Brands Of Oxytetracycline Capsules Available In Nigeria

[35] Agom JK, Akanni AO, Dawodu TO. QUALITY OF AMPICILLIN/CLOXACILLIN PREPARATIONS ON THE NIGERIAN.


[37] Yang YH, FU SG, PENG H, SHEN AD, YUE SJ, GO YF, YUAN L, JIANG ZF. Abuse Of

[38] Brown RC. Antibiotic Sensitivity Testing For Infections In Developing Countries: Lacking The Basics. JAMA. 1996 Sep 25; 276(12):952-3