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Journal Name:	**Journal of Advances in Medical and Pharmaceutical Sciences**
Manuscript Number:	**Ms_JAMPS_50886**
Title of the Manuscript:	**RESISTANT GENES OF MICROBES ASSOCIATED WITH ABATTOIR WASTES**
Type of the Article	**Original Research Article**

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty'**, provided the manuscript is scientifically robust and technically sound.
To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments	The paper "RESISTANT GENES OF MICROBES ASSOCIATED WITH ABATTOIR WASTES" has discussed the initial study of genes involved in multi-resistance. The paper has many problems which stated below before publication such point must be taken care. Major Revision: More study required. Errors in Methods. A fraction of beta-lactamases has studied. 16S sequencing data not presented. blaTEM, blaSHV and blaCTX-M must be sequenced to confirm PCR data. **Specific points to consider revision:** **Summary** **The presence of Extended Spectrum β-lactamase genes was checked for in the multidrug resistant isolates after they had been identified using genomic techniques. Two strains of *Escherichia coli* had the CTX-M gene, *Pseudomonas* sp. strain 6174 had the SHV and TEM genes, *Bacillus amyloliquefaciens* had the SHV gene, *Bacillus flexus* had the TEM genes, *Staphylococcus aureus* had SHV and TEM genes, *Proteus mirabilis* had the CTX-M and TEM genes while *Klebsiella* sp. strain EIKU11 possessed all three resistance genes.** The presence of Extended Spectrum β-lactamase genes was checked for in the multidrug resistant isolates after they had been identified using genomic techniques. What you mean by genomic techniques? You must say Beta-lactamases , not ESBL, as TEM and SHV are not ESBL. Beta-lactamases are denoted by *bla* gene, Hence there is no TEM, SHV and CTX-M gene unless you write *bla*TEM, *bla*SHV and *bla*CTX-M. So you have to correct where *bla*CTX-M is ESBL. You have not checked the deadly genes *bla*OXA and *bla*NDM which could inactivate the carbapenem drug like doripenem and meropenem which are used now to cure MDR infections. Such question is important as 40% resistant due to blaTEM reported in the environment where 0.002% now for carbapenem. When 100x increased to 0.2%, (likely after15-30 years) we and animal will face danger. Many peoples will be dragged to poverty line due to high cost of gene medicines ! See the references and add in the discussion that heterogeneities are so prominent, all publications are fractional story of multi-resistance and drug void. *van Hoek AHAM, Mevius D, Guerra B, Mullany P, Roberts AP and Aarts HJM (2011) Acquired* *antibiotic resistance genes: an overview. Front. Microbio. 2:203. doi: 10.3389/fmicb.2011.00203* *Chakraborty AK. (2016) Multi-drug resistant genes in bacteria and 21st Century problems associated with antibiotic therapy. Biotechnol Ind J.* ***12****(12): 114.* *Chakraborty AK, Pradhan S, Das S, Maity M, Sahoo S, Poria K. (2019) Complexity of OXA Beta-Lactamases involved in Multi-Resistance. British J Bio-Medical Res.* ***3*** *(1): 772-798. Doi: 10.24942/bjbmr.2019.424.* *Klebsiella* sp. strain EIKU11 possessed all three resistance genes but not *E. coli* is a wrong statement.	

Revise the statement and you can say, blaTEM, blaSHV and blaCTX-M genes were found in many Gram(-) bacteria where Klebsiella species showed prominence of all three beta-lactamases.

Introduction

Antibiotics resistance however, refers to the mechanism by which microorganisms become resistant to an antibiotic which include deterioration of the antimicrobial substance, modification of the enzymatic structure of the antibiotic, over-secretion of the target enzyme, obtaining alternate pathways to those drugs that can inhibit or cause changes in the bacterial cell permeability restricting the access of the antimicrobial agent to the target site, active removal of the antibiotic from the bacterial cell and remodeling of the target for the antibiotic.

What is mean by deterioration of the antimicrobial substance?
What is mean by modification of the enzymatic structure of the antibiotic? Antibiotic is a chemical not enzyme !

Delete the paragraph.

Material and Method

The PCR mix was made up of 0.4 M of pimers, X2 Dream taq Master mix (Inqaba, South Africa) and the template which was the extracted DNA. The Master mix was made up of Magnesium Chloride (MgCl), taq polymerase and DNTPs (Deoxyribonucleotides). The conditions for the reaction were Initial denaturation at 95 °C for 5 minutes, denaturation at 95 °C for 30 seconds, annealing at 52 °C for 30 seconds, extension at 72 °C for 30 seconds and final extension at 72 °C for 5 minutes.

O.4M primers?????? 0.4nM
taq polymerase?? Taq DNA polymerase
MgCl?? $MgCl_2$
DNTPs?? dNTPs

Sizes of resolved products were 281 bp, 560 bp and 960 bp for SHV, CTX-M and TEM, respectively.

For 960bp, 30" /72^{0}C extention is risky??? Explain
The resultant product was resolved on a 1 % agarose gel at 120V for 25 minutes.
120V will melt your minigel.
Give company name and specification of gel electrophoresis apparatus!!!!!!!

CTX-MF primer is CTX-M-1/3/15/52/57/79/114 type(BLAST indicated) but other type of major CTX-M family beta-lactamases are CTX-M-2 and CTX-M-9. You your story is partial.
Discuss the authenticity of your result in the light of multiplicity of beta-lactamases types with millions of isomers where many may be excluded as you found few genes in *E coli*.
If you made primers indicate the software you used and if obtained from papaers indicates the papers.

In figure-2 Aug resistant are major, then how you will not get blaTEM??????

	Ciprofloxacin sensitive data is important indicating no gyrA mutations!!! Check it Table-3 Klebsiella pneumonia has no mdr genes but in summary you told it has three genes.?? Please remove the table!! **<u>Result</u>** **<u>Discussion</u>** **Microorganisms isolated in this study were of the genus *Staphylococcus, Pseudomonas, Klebsiella, Proteus* and *Bacillus.* Similar organisms had been isolated from abattoir environments by some authors ([35]; [36]; [37]; [38]).** Ref [35-38] **The resistance genes checked for in this study were CTX-M, SHV and TEM which are genes coding for the production of extended spectrum β-lactamases- enzymes able to deactivate extended spectrum β-lactam drugs, which are 3rd and 4th generation cephalosporins [26].** Wrong statement. Only beta-lactamases early (blaTEM and blaSHV) and ESBL (CTX-M) but we have not studied deadly beta-lactamases like blaKPC, blaOXA and blaNDM beta-lactamases. Why you have not give plasmid profiles as stated by ref 35, ref 38 and ref 43 from your country. You must gave few c16 sequence data by compare of a part of chromatogram. What is new in your paper, discuss. Please include in the discussion: Gene involved in phosphotransferases (aph, str) and acetyltransferases (cat, aac) and adenyltransferases (ANT) are also predominant in plasmids causing multi-resistance but we are planning to do such study.	

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Minor REVISION comments		
Optional/General comments		

PART 2:

	Reviewer's comment	**Author's comment** ***(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)***
Are there ethical issues in this manuscript?	***(If yes, Kindly please write down the ethical issues here in details)***	

Reviewer Details:

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