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Journal Name:	British Microbiology Research Journal
Manuscript Number:	2014_BMRJ_12342
Title of the Manuscript:	A cross sectional study on prevalence of antibiotic resistance and efflux pump inhibition of isolated cultures from poultry, dairy farms and MTCC strains from reservoirs
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.

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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 manuscript lacks a clearly-stated and properly-developed hypothesis. Thus, the authors must present a clear and well-developed hypothesis (with a logical rationale), both in the abstract and in the introduction. Lines 90-92. The authors are lacking details needed to know how they identified their bacterial samples. Thus, appropriate details are needed here. Lines 93-102. For the drug susceptibility determination, the authors should include the interpretation threshold values for determining whether the bacterial samples are considered drug susceptible, resistant or intermediate. Lines 112-118. Regarding the determination of the drug MICs, the authors should use the standards set forth by the CLSI and cite the appropriate published protocol. Importantly, the authors did not include any negative control strains that lack drug efflux pumps. Therefore, it is virtually impossible to discern whether the inhibitors studied have any effect on strains with efflux pumps, since there are no controls to compare. It is critical that such important controls be included. The authors claim that piperine and plumbagin inhibit drug and multidrug efflux mediated bacterial resistance, but they did not actually measure drug efflux per se. This is the most serious drawback of the paper. Thus, the authors need to actually measure bacterial drug efflux activity directly using drug efflux assays in order to support their contention. Piperine alongwith ciprofloxacin acts as an EPI, Piperine and Plumbagin do not have activity of their own.	Done Done Done Done Present work was undertaken to screen variable resistance among bacterial samples obtained from various sources by using



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	Piperine and plumbagin are not direct efflux pump inhibitors; rather they collapse the membrane potential, which in turn is needed to drive the efflux pumps. That is, these agents affect respiration and energy coupling of the proton (ion) gradients to oxidative phosphorylation. Such biological effects will also affect other critical biological systems, such as growth, metabolism, etc. Therefore, these agents are indirect inhibitors of drug efflux pumps. Other studies that have used these agents actually studied drug efflux activity directly. Thus, the authors need to use agents that directly bind to and inhibit these drug efflux pumps or, as stated above, measure drug efflux directly. Since the Piperine and Plumbagin are indirect efflux pump inhibitors, So they were chosen as standards to check the possible availability of the efflux pumps in the bacterial samples. Direct evidence is lacking that any of the bacteria tested with the inhibitors actually have any efflux pumps. Readers will also be unsure which pumps are affected. The authors should, therefore, definitively identify which pumps are believed to be affected by the inhibitors. Further molecular biology studies need to be carried to definitively identify the possible pump responsible for the resistance. Our present study may support molecular biology studies.	minimal microbial techniques.
<u>Minor</u> REVISION comments	Line 42. The authors stated disease emergence has stimulated interest in generating new drugs against drug-resistant bacteria. This is completely untrue. The fact is that very few new drugs have been generated in the last 25 years. The authors should fix this inaccuracy. Line 46. The “threat to mankind” phrase is overstated and inaccurate. The statement should be re-stated so that it better reflects a threat to patients who have certain strains of pathogens with multi-drug resistant (MDR) properties. Thus MDR clinical pathogens are often recalcitrant to treatment with clinically used antibacterial agents. Done The authors chose 57 bacterial strains to study but neglected to state why they chose those particular organisms for study. The authors should state why they chose these ones. Along these lines the authors failed to state why they chose to study isolates from milk, chickens, buffaloes and nasal samples. This coincides with the lack of a rationale for their hypothesis. These all need to be adequately address in the paper. The samples were taken from variable sources to check if exposure to antibiotics (nasal samples from Poultry and milk samples from buffaloes) revealed more multidrug resistance as compared to reservoirs. This reviewer feels that once these additional experiments, controls, etc., are completed, the paper may be re-considered for publication.	
<u>Optional/General</u> comments		