The Clinical Significance of Gene Amplification in Heteroresistant Isolates

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Introduction:

The rapid emergence of antibiotic resistance is an increasingly major healthcare crisis (1). The Center for Disease Control and Prevention (CDC) estimates that annually over 2 million antibiotic resistant bacterial infections occur in the United States, resulting in at least 23,000 deaths (1). It is estimated that in the year 2050, 10 million deaths will occur annually due to infection by antibiotic resistant bacteria if no significant response is taken (2). In order to prevent this healthcare crisis from significantly worsening, we need to further our understanding of the antibiotic resistance mechanisms that bacteria use. One such resistant mechanism that is not very well understood is heteroresistance (3). Heteroresistance is a phenomenon where a subpopulation of bacteria are resistant to a particular antibiotic while the remaining population is susceptible (3). It is important to note that unlike persister cells, these resistant subpopulations are able to grow in the presence of the antibiotic (4,5).

The standard practices of identifying resistant isolates in the clinical setting, minimum inhibitory concentration (MIC) and disc diffusion, often times lead to a heteroresistant isolate being classified as susceptible (5–7). This is due to the small fraction of the population that is resistant to the antibiotic not being detected by these standard methods (6,7). It is believed that the misclassification of a heteroresistant isolate as susceptible may result in the administered antibiotic treatment failing. While this topic is still debated, increasing evidence shows that the presence of heteroresistant bacteria can result in treatment failure in the hospital setting(7–11).

Although heteroresistance is an important phenomenon to understand due to the potential of clinical misclassification resulting in treatment failure, the mechanisms are still not well understood (5). In this review, we will first emphasize the need to better understand heteroresistance. Subsequently, we focus on recent findings that highlight how tandem gene amplification enables bacteria to have subpopulations of resistance bacteria.

The Prevalence and Clinical Relevance of Heteroresistance:

Heteroresistance was first described in 1947 (12). Since then, this phenomenon has been reported in important pathogenic bacteria such as *Acinetobacter baumannii*, *Mycobacterium tuberculosis*, *Streptococcus pneumonia*, and *Enterobacter cloacae* (8,13–15). Additionally, heteroresistance has been shown to occur with the last line of defense antibiotics—aminoglycosides, carbapenems, and colistin (10,15,16). The golden-standard for detecting heteroresistance is population analysis profiling (PAP) (5). However, this method is too laborious to be used in the clinical settings, even though the techniques that clinical laboratories in hospitals use to determine a bacteria's susceptibility to antibiotics fail to identify heteroresistant bacteria (5).

The inability of hospital clinics to accurately determine the susceptibility of antibiotics on heteroresistant bacteria isolates is quite concerning. The improper classification of susceptibility is emphasized by the recent work of Edgar et al. through PAP of 108 strains of carbapenem-resistant *A. baumannii* (CRAB) isolates. The clinical determination for these isolates was that 60.2% (65) were susceptible to tobramycin while 39.8% (43) were resistant. However, PAP determined that 56.5% (61) were susceptible, 7.4% (8) were resistant, and that 35.8% (39) were heteroresistant to tobramycin (17). These results highlight the inability to accurately detect heteroresistance in the clinical setting; however, they do not show that this misclassification of susceptibility leads to treatment failure.

A case study centered around an infection of *A. baumannii* showcases how misclassification of susceptibility may lead to treatment failure. An E-Test was performed on an isolate stemming from a CSF sample, and colistin was determined to be an effective treatment (fig 1A). Despite an E-Test demonstrating that this isolate was susceptible, the treatment proved to be ineffective. A CSF sample was collected five days after the administration of colistin and once again an E-Test was conducted. However, this time, the E-Test showed that the bacterial infection was resistant to colistin (fig 1B). The initial E-Test did not detect the subpopulation of bacteria that were resistant to colistin. After treatment with colistin for five days, the resistant subpopulation was the only remaining bacteria and it continued to cause an infection in the patient. The inability to identify the colistin-resistant subpopulation led to inappropriate use of the antibiotic, which resulted in an ineffective treatment for the pathogenic infection (9). This case study demonstrates the danger of misdiagnosing a heteroresistant isolate as susceptible. However, as previously stated, clinical hospitals are unable to detect these

resistant subpopulations. One of the reasons heteroresistance is so difficult to detect through standard clinical methods is that the resistance of these subpopulations of bacteria results from tandem gene amplification, which is oftentimes unstable.

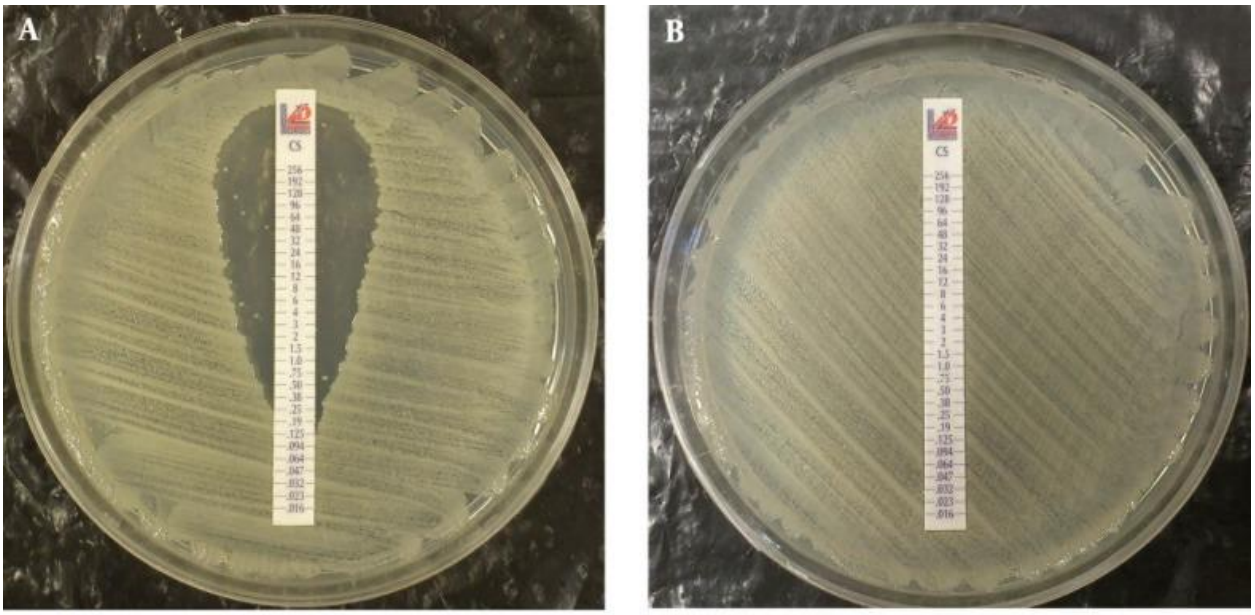

Figure 1: E-Test performed on two sputum samples before and after antibiotic treatment

(A) Results of an colistin E-Test from the first collected isolates of *A. baumannii*. (B) Results of an colistin E-Test of an isolate collected after treatment with colistin (9).

Tandem Gene Amplification in Heteroresistance Isolates:

Tandem gene amplification generally occurs through a genetic transfer between two homologs by way of homologous recombination (18). The exchange can result in a genome increasing its copy number of a specific gene or several genes. Usually, a higher copy number in bacteria will cause there to be an increased expression of the gene. The increase in amplification is highly unstable and has a large fitness cost. Because of this, the bacteria often revert to a lower copy number when they are not experiencing a selective pressure that encourages a higher copy number of the particular gene (18).

For some heteroresistant isolates, a large population of monoclonal bacteria will have a subpopulation of bacteria where homologous recombination has occurred and their gene number for an antibiotic resistant gene

has increased (5). The resistant gene alone is not enough to grant resistance. However, the increased amplification of the gene will cause the subpopulation of bacteria to be resistant. If this bacteria population were to be introduced to a specific antibiotic, the susceptible population, due to lack of amplification of the resistance gene, will be eliminated. The subpopulation whose copy number of their resistance gene has been increased will be able to survive and reproduce. However, in the absence of this antibiotic, the surviving population, over time, will see a decrease in their amplification of this gene and revert to being susceptible (17).

While it is well understood that amplification of resistant genes can result in heteroresistance, there is uncertainty of why amplification of specific regions is occurring. It has been hypothesized that inverted repeats may be responsible for why there is specific regions in the genome that are being amplified. However, this hypothesis has yet to be well studied. We must improve our understanding of the mechanism of heteroresistance so we can better detect its occurrence in the clinical setting and learn more about how bacteria are evolving to become antibiotic-resistant.

Conclusion:

Heteroresistance proves to be a unique and crucial phenomenon that can go undetected in the clinical setting. Although it has been shown that these subpopulations of resistant bacteria can result in treatment failure in both animal models and case studies (7–11,19,20), a thorough study on the association between infection by a heteroresistance pathogen and mortality and morbidity has not been done. A study of this nature is necessary for us to gain a better idea of how heteroresistant bacteria escaping identification impacts treatment care.

While this review has mainly focused on how infection by a heteroresistant bacteria can result in treatment failure, a recent study has shown that isolates that display heteroresistance to two or more classes of antibiotics can effectively be treated with two of these drugs (21). The study demonstrated that previously thought of pan-resistant isolates were heteroresistant to several types of antibiotics and that combination therapy with these antibiotics in mice models resulted in successful treatment (21). These findings, alongside the possibility that the misdiagnoses of heteroresistant bacteria may result in treatment failure, stress the seriousness of being able to detect heteroresistance in the clinical setting. Not only does misdiagnoses potentially lead to

antibiotic therapy failure, but it may also result in the missed opportunity to identify a successful treatment to multi-drug or pan-resistant isolates. With the continual rise in antibiotic resistance infections, it has become more important than ever to develop a method to detect heteroresistance in the hospital setting.

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