Review Article

Antibiotic resistance in prevalent bacterial and protozoan sexually transmitted infections

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Abstract

The emergence of multi-drug resistant sexually transmitted infections (STIs) is causing a treatment crisis across the globe. While cephalosporin-resistant *gonorrhea* is one of the most pressing issues, extensively antibiotic resistant *Chlamydia trachomatis* and *Mycoplasma hominis* are also becoming commonplace. Experts have suggested that the failure of current treatment regimens are "largely inevitable" and have called for entirely new classes of antimicrobial agents. With the exception of several new classes of drugs primarily targeting nosocomial infections, progress has been slow. While pharmaceutical companies continue to introduce new drugs, they are based on decade-old discoveries. While there is disagreement about what constitutes new classes of antibiotics, many experts suggest that the last truly new family of antimicrobials was discovered in 1987. This review summarizes the existing literature on antibiotic resistance in common bacterial and protozoal STIs. It also briefly discusses several of the most promising alternatives to current therapies, and further examines how advances in drug delivery, formulation, concentration, and timing are improving the efficacy of existing treatments. Finally, the paper discusses the current state of pharmaceutical development for multidrug-resistant STI.

Key words: Antibiotic, bacteria, protozoa, resistance, sexually transmitted infections

INTRODUCTION

While antimicrobial resistance in sexually transmitted infections (STIs) is not new, the emergence of multi-drug resistant strains is a relatively recent phenomenon. For instance, tetracycline-resistant *Neisseria gonorrhea* was first reported in 1980's,[1] and ciprofloxacin-resistance, in the early 2000's.[2] Now, according to the Centers for Disease Control and Prevention (CDC), with 334,826 reported cases of *gonorrhea* in the United States in 2012, 33.4% of sampled isolates were resistant to penicillin, tetracycline, ciprofloxacin, or some combination of these. In addition, 1% were cefixime-resistant; 14.7% ciprofloxacin-resistant; and 0.3% azithromycin-resistant. Growing antimicrobial resistance has also been observed in syphilis;[3] *Chlamydia trachomatis*;[4] *Trichomonas vaginalis*;[5] *Haemophilus ducreyi*;[6] and *Mycoplasma genitalium*.[7,8]

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The prevalence of multi-drug resistant STI varies widely across different regions of the world, but some of the highest levels have been found in low and middle-income countries.[9-13] The reasons are complex but include poor-quality health services, a high burden of infectious disease, less regulatory oversight, inappropriate dosing and use

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of antimicrobials, and low knowledge about the risks of microbial resistance. Not far behind however, are industrial countries that are also contributing to the growing STI resistance through overuse of antimicrobials, fortifying animal feed and plant fertilizer with antibiotics, and use of mono-therapy where multi-drug regimens would better preserve the current drug armamentarium.

ANTIBIOTIC RESISTANCE IN PREVALENT BACTERIAL AND PROTOZOAN SEXUALLY TRANSMITTED INFECTIONS

Neisseria gonorrhea

According to the most recent estimates, about 106 million adults are newly infected with *Neisseria gonorrhea* annually.[14] The organism has shown a remarkable ability to resist antibiotics; it developed resistance to sulfonamides in the 1940's, penicillin by the late 1970's, and fluoroquinolones in the 1990's. More recently Tetracycline-resistant strains have been found across the world.[15] Most worryingly, resistance to the last class of pharmaceuticals useful in *N. gonorrhea* monotherapy, extended-spectrum cephalosporins (ESC), is now being reported worldwide.[16-18]

There are a few alternative treatment alternatives for *N. gonorrhea* infections with high-levels of resistance to ESC. Options include the use of Spectinomycin, gentamicin and azithromycin although each of these have drawbacks including limited availability, high cost, and poor bioavailability at some anatomical sites.[19-21]

Syphilis

The World Health Organization (WHO) estimated that there were 10.6 million new cases of syphilis in 2008 with the majority occurring in developing countries. Reports of resistance to macrolides, the main alternative to penicillin for treating *Treponema pallidum* infections, started in the 1960's. South *et al.*, and Fenton and Light reported syphilis treatment failure using erythromycin as early as 1964.[22,23] Reports followed of resistance to azithromycin,[24] clindamycin,[25] and rifampin.[26] While there is currently concern that *T. pallidum* may develop resistance to tetracyclines and its derivatives, so far there is little evidence this is happening.[27,28]

Chlamydia trachomatis

The WHO estimated that there were 105.7 million new cases of *C. trachomatis* genital infections worldwide in 2008.[14] There is currently little data on the prevalence of chlamydia strains resistant to tetracyclines, macrolides, and fluoroquinolones, the primary pharmacologic treatment options, but there are a growing number of reports of unacceptably high recurrence rates in sexually active populations.[29] Until recently it was assumed that failures were the result of re-infection but that has now come into question.[4,29] Several studies have shown azithromycin treatment failure among both women and men with chlamydial infections who were not at-risk for reacquiring the bacterium.[30,31]

Several studies have documented *C. trachomatis* isolates that were found to be resistant to one or more drugs including doxycycline, azithromycin, ofloxacin, tetracycline, erythromycin, sulfamethoxazole and clindamycin.[32,33] The prevalence of these strains is unknown but given the widespread nature of the bacteria and sequelae including pelvic inflammatory disease (PID), infertility, ectopic pregnancy, and chronic pelvic pain, there is a compelling need for more research into the mechanisms of antimicrobial resistance.

Trichomonas vaginalis

Trichomoniasis is the most prevalent STI in the world.[14] Caused by the protozoa *T. vaginalis*, it was estimated in 2005 to affect about 250 million adults aged 15–49. For the last 40 years, metronidazole has been the gold standard for treatment of *trichomoniasis*. Recently, however, evidence of widespread metronidazole-resistant trichomonads has been emerging. This has raised serious public health concerns since vaginal *T. vaginalis* infection has been associated with increased risk for HIV-1 acquisition, adverse pregnancy outcomes, and PID.

A study carried out in the United States found that 4.3% of *T. vaginalis* isolates exhibited low-level resistance. The highest prevalence of resistance was found in Denver (7.5%), Seattle (5.8%) and San Francisco (4.9%).[5] Metronidazole-resistant trichomonads have also been observed in Finland,[34] Iran,[35] Spain,[36] and Papua New Guinea.[37] In addition, a study of samples sent to the U.S. Centers for Disease Control and Prevention from United Kingdom, South Africa, Australia, and India also showed from low to high resistance to metronidazole.[38]

Haemophilus ducreyi

Haemophilus ducreyi is the organism that causes Chancroid, an STI that is common in developing countries in Asia, Africa, and the Caribbean. The WHO estimates that approximately seven million people are newly infected with the bacterium each year. Like *T. vaginalis; H. ducreyi* also increases risk of HIV acquisition.

There are surprisingly few susceptibility or prevalence studies of *H. ducreyi*. A review by Dangor *et al*., identified most of the available literature.[39] Research has shown that the *H. ducreyi* bacterium produce high levels of ß-lactamases making them resistant to penicillins, cephamycins, and carbapenems.[39,40] Strains have also been identified treatment resistant to erythromycin, kanamycin, chloramphenicol, trimethoprim, and tetracycline.[39,41] Unfortunately, almost all studies on *H. ducreyi* susceptibility are from the 1980's, and little is known about the present prevalence of *H. ducreyi* drug-resistant strains.

Mycoplasma genitalium

Mycoplasma genitalium has only recently been recognized as a sexually transmitted pathogen because due to its fastidious nature and the difficulty of culturing it in the laboratory. With the advent of polymerase chain reaction-based detection methods, there has been growing interest in trying to understand the role of *M. genitalium* in urethritis, cervicitis, and PID.[42] This has given recent impetus to drug susceptibility studies to better understand the effectiveness of various treatments. There is growing evidence that use of tetracyclines leads to high rates of treatment failure, so current strategies focus on the use of macrolides like azithromycin, quinolones like moxifloxacin. Ciprofloxacin, levofloxacin and ofloxacin all show evidence of declining efficacy.[42] Recent studies have shown an alarming increase in *M. genitalium* with genotypic markers of macrolide and fluoroquinolone resistance among men with urethritis. In addition, moxifloxacin-resistant bacteria were identified in Australia,[43] and another study found about 60% of *M. genitalium* were resistant to azithromycin and 90–100% of men receiving the drug encountered treatment failure.[44]

Pharmaceutical industry response

Antibiotic resistance has arisen over the last several decades, but few new antimicrobial drugs have been developed in that time. Between 1930 and 1962 the pharmaceutical industry introduced 20 new classes of antibiotics. Since then, only four novel drug classes have been added to the armamentarium, and most new products are derivatives of existing drugs. Many of these have lost efficacy as bacteria developed cross-resistance to entire classes of antibiotics. In the last decade, only one new class of antibiotics, the lipoglycopeptides, and the repurposed anticonvulsant drug lamotrigine (not yet been approved for use as an anti-microbial) utilize completely new antibacterial mechanisms. Among the 13 antibiotics approved by the Federal Drug Administration since 2004, none are approved for the treatment of STI. The last drugs to find general use in these infections were introduced in 1993.

According to a 2014 Pew Trust report, there are currently 38 antibiotic drugs in the development pipeline. Of these 11, 18 and 7 are in Phases I, II, and III clinical trials, respectively, and two are recent new applications. According to the analysis by the Independent Institute, it usually takes about 7–13 years from application to approval by the US Food and Drug Administration. Among applications submitted, only about 8% reach the market. Their analysis suggests a conditional rate of 75%, 48%, and 64% for each of the three clinical trial phases. In other words, we can expect about 18 drugs to eventually be approved; with the first of these approvals in about 3 years, and the remainder over the next decade. How "Generating Antibiotic Incentives Now" (GAIN) will affect the length of the approval process is not yet known; it may shorten the development cycle but will do little to impact approval rates. Recognizing that there was a dire shortage of effective antimicrobials and few new drugs in the pipeline, the U.S. Congress passed the GAIN Title of the Food and Drug Administration Safety and Innovation Act, and President Barack Obama signed the measure into law on July 9, 2012. Under GAIN, pharmaceuticals designed to treat serious or life-threatening infections, particularly those caused by drug-resistant pathogens, can now be designated as "Qualified Infectious Disease Products" (QIDPs), which are eligible for an expedited development pathway and priority review. In addition, QIDPs receive 5-year free from generic competition in addition to any existing other regulatory benefits for which they would have been eligible.

ALTERNATIVE STRATEGIES FOR TREATING ANTIBIOTIC RESISTANT PATHOGENS

The prospect that the fast pace of growing bacterial resistance will completely overtake drug development is becoming more likely each day. Such a prospect is almost unimaginable since most medical procedures depend on some type of antimicrobial cover; surgery, emergency medicine, chemotherapy, and even cesarean sections will become procedures with unacceptably high mortality. Finding alternatives for antibiotics has become a critical public health need but there has been little tangible progress. Researchers are currently exploring several nontraditional approaches for

treating antibiotic resistant infections; these include bacteriophage therapy, antimicrobial peptides (AMPs), phytochemicals, metalloantibiotics, Efflux Pump Inhibitors, and a variety of other strategies for interfering with biosynthesis proteins needed for bacterial metabolism and propagation.

Phage therapy

Bacteriophage or viruses that attack and infect bacteria have been used in medicine for almost a century. The originator of phage therapy, French-Canadian microbiologist Felix d'Herelle, was the first to realize their potential for treating bacterial infections.[45] Starting in 1917 he used bacteriophage to treat a wide range of pathogenic infections including *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli*, *Pasteurella multocida, Vibrio cholerae, Yersinia pestis*, *Streptococcus* species, *Pseudomonas aeruginosa* and *Neisseria meningitis*. While phage therapy was immensely popular in the 1920s and 1930s, interest in their use waned after the introduction of antibiotics. Even though phage therapy was abandoned in most parts of the world; Russia, Poland and Georgia continued using them in both research and therapy through the present day.

Currently, research has focused on the use of bacteriophage for delivery of antimicrobial agents to infection sites, as adjuvants for bactericidal antibiotics and as treatments antimicrobial resistant organisms. While much of the work has been focused on antibiotic-resistant nosocomial infections, several research teams are examining the use of phages for management of STI. Bhattarai *et al*., at the University of California, Berkeley has demonstrated the use of engineered phages to inhibit *C. trachomatis* intracellular infection.[46] Lovett engineered bacteriophage vectors containing recombinant DNA derived antigens of *T. Pallidum* for propagation within *E. coli*; a potential immunizing agent against syphilis;[47] and Piekarowicz *et al*., has conducted bioinformatic analysis of the genome sequence of *N. gonorrhea* identifying prophage islands that encode functionally active phages.[48]

Antimicrobial peptides

A growing body of research has demonstrated the importance of AMPs as part of the innate immune system in all living organisms. Over the last several decades, there has been interest in harnessing these effector molecules found in bacteria, plants, and animals as antimicrobials. The bactericidal action of most AMPS involves disruption of bacterial membranes and binding to intracellular molecules to inhibit cell wall biosynthesis and DNA, RNA, and protein synthesis. While research is still preliminary, several studies suggest that they have promise in the treatment of STI. Zairi *et al*., have demonstrated anti-gonorrheal effects using synthetic peptides derived from the natural AMP dermaseptin S4.[49] Research has shown that the LL-37 AMP had broad-spectrum activity against a number of pathogenic bacteria including *T. pallidum* and Protegrin AMP found in porcine leukocytes had bactericidal effects against *N. gonorrhea*, *C. trachomatis, H. ducreyi* and even HIV.

Phytochemicals

The use of phytochemicals or bioactive compounds found in plants for treatment of bacterial infections has been documented from time immemorial. Extracts for instance, from *Caryophyllus* aromaticus and *Syzygyum* joabolanum have been shown effective in controlling a wide range of bacteria including those resistant to current antibiotics. Current research is focused on phytochemical adjuvants for other antibiotics; as chemotherapeutic agents and for use against bacterial biofilm. While ethno-biologists have documented use of medicinal plants such as *S. cyaenneusis* in the treatment of STI such as syphilis, and *gonorrhea*, their efficacy remains largely untested.

Other approaches

Research is also ongoing on several other approaches to antibiotic resistance including the addition of metal ions to existing antibiotics such as tetracyclines, aureolic acids, and quinolones. This approach appears to revitalize certain antibiotics to which bacteria are already resistant, but its use appears limited due to side effects and high toxicity. Other researchers are examining the use of agents that attack bacterial defenses such as efflux pumps, and Lipopolysaccharide Layers, or inhibit basic bacterial functions such as the transcription of DNA into RNA or protein synthesis. Much of the current STI-related research has focused on finding alternatives to treat multidrug-resistant, extensively drug-resistant and untreatable *gonorrhea*. Studies are currently evaluating the efficacy of efflux pump inhibitors, host defense peptides such as LL-37, and agents that inhibit outer membrane biosynthesis by targeting LpxC, a single copy gene found in most Gram-negative bacteria.

CONCLUSIONS

As Fauci and Marston have noted, antimicrobial resistance is a perpetual challenge; a persistent struggle for dominance.[50] Antibiotic resistance, a natural consequence of evolutionary selection, has been accelerating in recent decades due to antibiotic

overuse and misuse abetted by international travel, commerce, and the spread of disease. The current crisis is a perfect storm of neglect, inattention to the repeated warnings from researchers and clinicians about antibiotic resistance dating to the early 1960s, and shifting pharmaceutical industry priorities that deprioritized antibiotic research and development. The culmination of all these factors is now estimated to cause about 23,000 deaths in the U.S. and 25,000 each year in Europe along with untold thousands in low and middle-income countries.[50]

It is still early in the exploration of novel strategies for treatment of antibiotic resistance. The discovery of multidrug and extensively drug-resistant forms of *gonorrhea* has given the search new urgency, but usable products are decades away. The current drug pipeline also gives little reason for optimism since it contains few novel antibiotics, and most applications are at early clinical trial stages. There are also few alternatives; even phage therapy faces considerable obstacles because of the historical bias toward the use of antibiotics in the western world and the lack of required infrastructure. The best we can hope for it seems is a slowing of antimicrobial resistance; a poor prospect indeed.

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