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Case Study

Wound Infection with Multi-Drug Resistant *Clostridium Perfringens*: A Case Study

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Abstract

Wound infections are among public health problems worldwide. However, progress has been made in improving surgical techniques and antibiotic treatments. Misuse/overuse of antibiotics to prevent and treat bacterial infections eventually leads to increased bacterial resistance with rising incidences of multi-drug resistant (MDR) bacterial strains. The wider dissemination of antibiotics may ultimately result in ineffectiveness to antibiotic therapy, thereby complicating/graving the outcome of a patient. In the present study, a 60-year-old male patient having wound infection with MDR bacterium that ultimately required surgical amputation of the toe was investigated. For the confirmation of MDR bacterium, two culture media viz., MacConkeyAgar and Mueller Hinton Agar media were used. The sensitivity of the isolated strain for various antibiotics was tested using the disc diffusion method. The wound sample was found positive for Gram-positive bacterium that was identified as *Clostridium perfringens*. The bacterium was screened for 40 antibiotics, and among all the antibiotics, it was found sensitive for only Piperacillin/Tazobactam antibiotic combination. *C. perfringens* bacterium caused the gas gangrene in the infected wound part of the patient. Amputation of the gangrene – affected foot part was performed by surgery, and with good medical care, the person recovered fast. To the best of our knowledge, this is the first-ever report of MDR*C. perfringens* single isolate harboring resistance against at least 40 antibiotics tested. More research is needed to develop really new and effective medicines that do not cross-react with antibiotics now in use and have robust activity against MDR organisms.

Keywords: *Clostridium perfringens*, Gas gangrene, Multi-drug resistance, Wound

1. Introduction

Emerging incidences of antimicrobial resistance and especially the life-threatening bacterial infections are posing high public health concerns at the global level by lessening the outcomes of anti-bacterial treatment, increasing morbidity and mortality, elevating the cost of treatment, and creating a high burden on the health care system. The unethical and indiscriminate use of synthetic antibiotics for prophylaxis and treatment of numerous bacterial illnesses has been a source of the appearance of multi-drug resistance (MDR). Fora long, it has been heavily criticized due to an increase in the number of drug-resistant bacterial strains that are resistant to a variety of antibiotics used to treat various bacterial diseases (1). Infections with MDR bacteria have created substantial implications on the clinical outcomes of the patients by heightening the levels of bacterial resistance which is mostly non-treatable with available antibiotic drugs that ultimately lead to more deaths. Therefore, besides searching to find out more effective and newer antibiotics for countering MDR bacterial strains (2-4), researchers and medical practitioners worldwide are searching out alternate therapeutic regimens for reducing the adverse impact of

bacterial pathogens especial to pull the brakes on fast and furious MDR strains (1,5-8).

The MDR bacteria are becoming more common in both acute and chronic wound infections. Despite advances in surgical techniques, antibiotic prophylaxis application and wound infection management remain major public health problems (9-11). Excessive use of antibiotics to prevent bacterial infections sometimes leads to increased bacterial resistance in wound infections and their dissemination. The wound creates a moist, warm, and nutrient-rich microenvironment in which infection can thrive. Bacterial burden, prolonged inflammation, and an imbalanced cellular defense mechanism are all common features of infected wounds.The common bacteria associated with wound infection are *Staphylococcus aureus*/methicillin-resistant *S. aureus, S.pyogenes, Enterococci, Pseudomonas aeruginosa*, and C*lostridiumperfringens* (12). Bacteria belong to the C*lostridium* genus are Gram-positive, spore-forming, rod-shaped, and anaerobic commonly found in the natural environment, such as soil and water. Among the species of this genus *C. difficile, C. perfringens, C. tetani, and C. botulinum*are ubiquitous pathogens associated with human and animal diseases (13). *C. perfringens*is an MDR bacterium and causes gas gangrene commonly known as clostridial myonecrosis and myonecrosis in both animals and humans (14-16). The illness of *C. perfringens* is associated with toxins producing bacterium at the infection site. Gas gangrene is associated with necrotic infection of soft tissue with high mortality rates in humans. Advanced age, malnutrition, hypovolemia, obesity, steroid usage, diabetes, steroid use, hypertension, smoking, coagulopathy, and concurrent infection in multiple organs are all risk factors for wound infection (17).

Here in the present study, we report a case of a 60-year-old patient suffering from wound infection due to MDR bacterium (i.e., *C. perfringens*) leading to the amputation of his toe. The antibiotic susceptibility pattern of *C. perfringens* isolated from the patient's wound was studied for the available 40 antibiotics (29 single+11 in different antibiotic combinations). The bacterium was found sensitive against only Piperacillin/Tazobactam antibiotic combination. Piperacillin has beta-lactamase activity, while Tazobactam has beta-lactam activity. Antibiotic and antimicrobial resistance has become a healthcare concern arising merely from the overuse and misuse of antibiotics.

2. Case Presentation

We report a case of a 60-year-old male patient having wound infection with MDR bacterium (i.e., *C. perfringens*) that leads to the amputation of the toe.

2.1. Chief Complaint

The patient was presented with complaints of blocking of the right little toe, pain, and swelling in the right foot, and general weakness from the past 15 days.

2.2. History of Present Illness

The patient's toe was wounded by a buffalo while milking the animal that caused a wound formation. Initially, the wound was cleaned with Betadineat a local clinic, and few antibiotics and pain killers were given to the patient. After two weeks, the pain enhanced and pus was also formed in the wound region; as a result, the patient visited the clinic with advanced medical facilities for examination and treatment.

2.3. Personal History

The patient was a farmer with a hardworking and active lifestyle. Before the incident happened, the patient used to do heavy works in the field and used to be on a vegetarian diet. He had no signs of distress. The patient was a smoker and had a history of chronic asthma. For chronic asthma, the patient was on medication. Apart from chronic asthma, the patient had no medical problems. The patient was able to take good care of himself. He had no history of hypertension, diabetes, or any other diseases.

2.4. Family History

No one in the patient family had the problem of hypertension, diabetes, or any severe diseases.

2.5. General Examination

Upon admission, the blood pressure was 130/90 mm Hg, his pulse was 90 min (RR-23 min), and he had normal body temperature. His heart showed a regular rate and rhythm without murmurs.

2.6. Investigation

For investigation, a complete blood count (CBC) and a pus culture sensitivity test were performed. In the CBC result, the hemoglobin was found at 9.0 g/dl, and red blood cellwas 3.37 mil/cm, both the values were less than the normal value. The platelet count was good, which was 2.14 lakh/cmm.

2.7. Microbiological Examination

As part of the regular microbiological procedures, the pus of the patient was cultured on MacConkey Agar and Mueller Hinton Agar (18) to identify the pathogen. The colonies were grown using the surface plating method, and the bacterium was identified based on the classical morphological/biological and chemical properties of Clostridium. Large, smooth, and convex colonies with a double zone of hemolysis and lecithinase production were the basis for the identification, and the colonies were identified to be positive for a Gram-positive bacterium (i.e., *C. perfringens*).

An antibiotic sensitivity test was conducted using a panel of 40 antibiotics (Table 1). Out of 40 antibiotics examined separately or in combination, the bacterium was found to be sensitive to just the antibiotic Piperacillin/Tazobactam combination.

Table 1. A list of antibiotic sensitivity test

S. No	Antibiotics	Abbreviations	Group	Sensitive (S)/Resistant (R)
1	Aztreonam	AZT	Beta-lactam	R
2	Azithromycin	AZI	Macrolides	R
3	Cefepime	CEP	Cephalosporins	R
4	Cefotaxime	CET	Cephalosporins	R
5	Ceftriaxone	CEA	Cephalosporins	R
6	Cefuroxime	CEO	Cephalosporins	R
7	Ceftazidime	CEZ	Cephalosporin	R
8	Ciprofloxacin	CIF	Quinolones	R
9	Clindamycin	CLD	Lincosamides	R
10	Cefexime	CEX	Cephalosporin	R
11	Cefoperazone	CEP	Cephalosporin	R
12	Imipenem	IMP	Carbapenem	R
13	Doxycycline	DOX	Tetracyclines	R
14	Erythromycin	ERY	Macrolides	R
15	Gentamycin	GEN	Aminoglycoside	R
16	Cefoxitin	CEF	Beta-lactam	R
17	Polymyxin b	POB	Polymyxins	R
18	Teicoplanin	TEI	Glycopeptides	R
19	Tetracycline	TET	Tetracycline	R
20	Ticarcillin	TI	Beta-lactam	R
21	Ofloxacin	OFL	Quinolone	R
22	Penicillin	P	Beta-lactam	R
23	Levofloxacin	LEF	Quinolone	R
24	Linezolid	LIZ	Oxazolidinones	R
25	Nitrofurantion	NIF	Sulfonamides	R
26	Norfloxacin	NOF	Fluoroquinolone	R
27	Netillin	NET	Aminoglycoside	R
28	Cloxacilin	CLO	Beta-lactamase	R
29	Colistin	COS	Polymyxins	R
	Antibiotics in combination			
30/31	Ceftazidime/Clavulanic acid	CCA	Cephalosporin/beta-lactamase	R
32/33	Ceftriaxone/Sulbactam	CES	Cephalosporin /beta-lactamase	R
34/31	Cefotaxime/Clavulanic acid	CCA	Cephalosporin/beta-lactamase	R
35/36	Cefoperazone /Tazobactam	CET	Cephalosporins/ Beta-lactamase	R
37/31	Amoxycillin/ Clavulanic Acid (Amoxyclav)	AMC	Beta-lactam/β-lactamase	R
38/39	Ampicillin/Sulbactum	AMS	Beta-lactam/β-lactamase	R
35/39	Cefoperazone/Sulbactum	CES	Cephalosporins/β-lactamase	R
36/40	Tazobactam/ Piperacillin	Tzp	Beta-lactamase/ beta-lactam	S

31-Clavulanic acid; 33-Sulbactam; 35-Cefoperazone; 36-Tazobactam; 39-Sulbactum (Antibiotics used in combination).

2.8. Diagnosis

The patient had a previous history of atherosclerosis and chronic asthma (19). Gas gangrene is a clinical condition after ischemia or infection characterized by necrotic tissue often circumferential around a digit or extremity. *C. perfringens* causes gas gangrene, and the morbidity of *C. perfringens* is linked with toxins produced by a bacterium (14-16). This organism, which is characterized by the presence of gas in subcutaneous tissue, can cause fast development of localized tissue necrosis and systemic indications of sickness in part due to its production of exotoxins. Additional bacterial infections can lead to gas production and infection spread quickly. Here, it appears to be the case of gas gangrene due to the infection developed in the wound by *C. perfringens.* The culture report indicated that the bacterium was widely resistant to many classes of antibiotics and was found sensitive towards only one antibiotic Piperacillin/Tazobactam among all the tested antibiotics.

2.9. Treatment

After the initial wound, the patient was treated with general antibiotics and pain killer. After some time, the infection developed prominently, and the patient was admitted to the hospital. Later, during the hospital stay, the patient was treated with medication and surgical treatment using intravenous antibiotics combination of Piperacillin/Tazobactam (4.5 grams) daily for 7days, with a supply of intravenous fluids, symptomatic, and supportive care. Color Doppler mode was used to identify inflammatory edema in subcutaneous fat and necrotic tissue of the infection site. Amputation of gangrene affected right foot was finally done. After surgery and proper medical care, the patient was recovered fast and the wound was healed completely (Figure 1).

Figure 1. Various stages of treatment and post-amputation images

3. Discussion

Wound infectionffigs remain among the public health problems in low- and middle-income countries despite the progress made on refining surgical techniques and applying proper antibiotic prophylaxis (9-11). The risk of wound infection in an individual depends upon various factors, including age, other diseases, and patient hygiene before surgery, post-surgical complications, and other medications with immunosuppressive therapies or wound colonization with the MDR bacterium, such as *C. perfringens.* Almost all bacteria can acclimatize to the present environmental conditions and resist drugs and antibiotics. The frequency of MDR bacteria is increasing day by day, and researchers are searching for effective and newer antibiotics, as well as alternative therapies to replace antibiotics usages (20).

Bacteria identified in wound infections most commonly derive from endogenous flora, which is favoured by invasive procedures or contaminating substrates used in the patient care.

The bacterium's MDR strains pose the greatest threat to public health. MDR bacteria are resistant to a wide range of treatments, including antifungal, antiviral, and antiparasitic medications (21). MDR in bacteria usually occurs by accumulation/modification of the multiple genes that encoded for a protein which gives resistance to a single drug, within a single cell. These accumulating/modifying genes are typically resistance plasmids, the genes for multidrug efflux pumps, antibiotic-modifying enzymes, reduction in outer membrane permeability, AmpC/porin reduction combinations, extended-spectrum β-lactamases, and mutant topoisomerases (22) which provide resistance against a wide array of drugs. These MDR plasmids are passed on to other bacteria, making them resistant to a wide range of antibiotics (23).

MDR may grow and spread further in circumstances where bacteria are constantly exposed to antibiotics, such as hospitals or factories where pharmaceutical medications are made, and unused/expired drugs are disposed without sufficient monitoring (24). The MDR may be acquired naturally, and antibiotic-resistant is very useful in antibiotic cycling. Antibiotic cycling is a tool used to reduce the occurrence of antibiotic resistanct bacteria in the intensive care unit (ICU) setting (25). This is a potential approach to reduce the emergence of antibiotic resistance. In antibiotic cycling, a class of antibiotic drugs is withdrawn to use for a definite time and reintroduced after a specific point of time to limit the bacterial resistance in ICU. Antibiotic cycling is a method to reduce the emergence of resistance that might occur as a result of using a single or limited number of antibiotic classes at a time (26).

MDR is a critical problem due to improper susceptibility tests, self-medication, and long duration of hospitalization. Infections with MDR bacteria are hard to treat since very few or even no treatment options are available. In some cases, due to MDR strains of the bacterium, healthcare providers are obligate to use antibiotics that are more toxic for the patient (27).

Here, we report a case of gas gangrene with very acute onset and rapid progression of the symptoms. This case report specifically focuses on finding an isolate of *C. perfringens* that displayed MDR against a wide range of antibiotics, and to the best of our knowledge, it is the first report of an exhibition of drug resistance against 40 antibiotics by a single isolate of *C. perfringens.*

The *C. perfringens* is a Gram-positive, spore-producing, anaerobic bacterium commonly distributed in nature and often found in the intestines of animals and humans as intestinal flora (28). *C. perfringens*requires an optimal temperature of 37ºC to grow abundantly (29). The bacteria *C. perfringens*is known for its MDR emergence with the persistence of both the toxigenic and non-toxigenic strains. *C. perfringens* is not commonly able to invade and multiply within healthy cells (28). There exist two mechanisms for the entry of this bacterium inside the

host cell. One is the oral route and another one is through wounds. However, its proliferation in the intestine or wounds depends on the presence of other factors, including diabetes, obesity, coronary artery disease, and malnutrition (29). After a traumatic injury, the bacteria is the most common cause of *clostridialmyonecrosis*, an acute and excruciating infection in which muscles and subcutaneous tissues fill with gas and exudate (29).

C. perfringens is a common etiological agent of gangrene. Dry gangrene, wet gangrene, gas gangrene, internal gangrene, and necrotizing fasciitis are the five kinds of gangrene illness. The necrotic tissue is infected in all cases, except for dry gangrene (30). Gas gangrene is a wound infection-related condition with a potentially fatal prognosis if treatment is delayed. Antibiotic medication and (if available) hyperbaric treatment led to the release of toxins in the body, causing shock, unconsciousness, and even death in the absence of early needed surgery. Due to the formation of toxins, this pathogen can be manufactured into biological weapons (1,31). It can be utilized to cause food poisoning epidemics and spore spread by missiles potentially increasing the morbidity and mortality of gas gangrene in injured soldiers (32).

C. perfringens was found in the majority of patients with gas gangrene in a prior investigation by Wang, Qian (33). Because of the low sensitivity of culturing techniques and the sampling of a small number of wound sites, the true prevalence of this organism may have been underestimated in the investigations (33). In Japan, a 54-year-old man with atherosclerosis obliterans was diagnosed with fulminant gas gangrene caused by *C. perfringens*. The patient's gangrenous lower limb was amputated after the revascularization of the iliac artery by endarterectomy (34).

From clinical and environmental perspectives, reduction of the bacterial exposure is a method for reducing the possibility of antibiotic resistance. Metronidazole is a drug that is used to treat *C. difficile* infections. Many researchers have reported *C. perfringens* infections. Stevens (35) indicated that the use of a combination of medications was more common in the treatment of *Clostridial* infection, and Metronidazole and Rifampicin were found to be more effective in the treatment of gas gangrene caused by *C. perfringens* (35,36). Stevens et al. studied the effect of various antibiotics (Penicillin, Tetracycline, Clindamycin, Rifampin, and Metronidazole) and found that Metronidazole and Rifampin kill the *C. perfringens* ATCC 13124 strain by rapid reductions in viability, turbidity, and alpha-toxin activity by 15 to 45 min (37).

The management of *Clostridium*infections is well-established now. The early and aggressive surgical debridement with extensive broad-spectrum antibiotic therapy should be performed. Simultaneously, close monitoring and physiological support are necessary for patient recovery (38). In this example, *C. perfringens* was identified as the source of sepsis in an atherosclerotic patient who was referred for surgical debridement as soon as possible. Tazact 4.5 gm containing Piperacillinand Tazobactam was given to the patient for 10 days after surgery. Iperacillin/Tazobactam is a beta-lactam antibiotic and beta-lactamase inhibitor. The combination of these two antibiotics is used to treat serious infections caused by Gram-positive, as well as against those organisms that develop extended-spectrum beta-lactamases.

To reduce the risk of MDR bacterial infection, various remedies and advanced treatment options have to be taken into the clinical practice, as well as the community environment. To prevent the infection, hygienic conditions need to be primarily maintained in the surrounding environment, and secondly, extreme caution could be taken in the administration of antibiotics given to an individual, preferably to be administered after analyzing antibiotics sensitivity and then the best acting antibiotic be chosen for the treatment.

Due to the less promising role of conventional therapy, researchers have been driven to investigate alternate treatments for MDR infections due to the rising prevalence of MDR bacteria. Recent studies suggested that nanoparticles (NPs) have a potential role

in the treatment of MDR strain since NPs efficiently interact with bacterium and alter the morphology and structure of bacterial cells which leads to the killing of the bacterium. Inorganic NPs, commonly gold NPs, have shown a potential role in the killing of microorganisms by various actions, such as generation of oxidative stress, inhibition of protein synthesis, modification of various essential enzymes, and inhibition of peptidoglycan synthesis that leads to the inhibition of cell wall formation (8).

5. Conclusion

The presence of a *C. perfringens* strain is commonly associated with soil-contaminated wounds and poor local outcomes in patients. Identification of *C. perfringens* is very difficult due to its presence in conjunction with various other organisms, including *P. aeruginosa, Bacillus cereus, Clostridium, Enterobactercloacae,* and *Acinetobacterbaumannii*. The treatment of *C. perfringens* infection is still extremely challenging, and therefore, most of the patients have unfavorable treatment outcomes even after getting multidisciplinary management. Accordingly, when a *C. perfringens* strain is identified in the patient from wound infection, an extensive and aggressive surgical excision should be urgently performed. Sometimes, there is a need for another surgery due to the presence of resistant spores of *C. perfringens* which are extremely resistant to most of the antibiotics. In the present study, a high level of multiple drug resistance was evident in *C. perfringens* that was identified in the patient. Due to the wound infection priority, very extensive and aggressive antibiotic treatment was given to the patient, followed by a surgical excision of wound infected part as bacterium was shown resistant for a broad class of antibiotics. This case report emphasized the close monitoring of pathogens harboring MDR, especially for those which are important in clinical settings. Efforts need to be put in a direction in taking measures to prevent the development of MDR in pathogens because the treatment is still quite difficult. To the best of our knowledge, this is the first report of *C. perfringens* isolate (obtained from the wound of a patient) encompassing resistance against a wide spectrum of 40 antibiotics.

Abbreviations

MDR: Multi-drug resistant; *C. perfringens: Clostridiumperfringens*; CBC: Complete blood count; ICU: Intensive care unit

Authors' Contribution

Study concept and design:
Acquisition of data:
Analysis and interpretation of data:
Drafting of the manuscript:
Critical revision of the manuscript for important intellectual content:
Statistical analysis:
Administrative, technical, and material support:

Ethics

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Barkatullah University, Bhopal, India.

Conflict of Interest

The authors declare that they have no conflict of interest.

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