



Surveillance of drug sensitivity of bacteria in skin ulcer infections

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Abstracts

Purpose: The bacterial manifestation in Skin ulcer is a common type of disease affecting patients' health and quality of life. Due to increases in antibiotic resistance, the difficulty of its management.

Methods: A prospective study was carried out on skin ulcers by collecting the results of bacterial culture sampled of 110 cases from January 2016 to December 2017 at our hospital. We analyzed the constituent ratios of ulcer surface bacteria, the change in the main infectious bacteria and the results of drug sensitivity testing for common bacteria. In addition, the characteristics of bacterial infection of skin ulcers were summarized.

Result: Out of 110 samples, 90 isolated bacteria were cultured. 61 samples were Gram-negative bacteria, mainly comprising *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli*. In addition, 23 isolates were Gram-positive bacteria, mainly comprising *Staphylococcus aureus* and *Enterococcus faecalis*. The probability of a negative bacterial culture in 2017 was significantly lower than that in 2016 (16.7% vs. 40.0%, $p < 0.01$). Moreover, the probability of *P. aeruginosa* infection in 2017 was significantly higher than that in 2016 (31.7% vs. 14.0%, $p < 0.01$). *P. aeruginosa* was resistant to seven commonly used antibiotics. Both *K. pneumoniae* and *E. coli* had higher resistance to ampicillin. *E. cloacae* were not sensitive to piperacillin/tazobactam. *Acinetobacter baumannii* was resistant to all the tested drugs. *S. aureus*, *E. faecalis* and *Staphylococcus epidermidis* had high resistance to clindamycin. There was other drug resistance to reflect the higher rate of skin bacterial resistance.

Conclusion: Skin bacterial resistance rate is high. Gram-negative bacteria gradually account for the majority, and *P. aeruginosa* becomes the most important skin infection pathogen. These characteristics of bacterial infections of skin ulcers provide a significant reference for guiding the selection of antibiotics, better controlling infections of skin ulcers and accelerating the healing of skin ulcers.

Keywords: Skin ulcer, Infection Bacteria, Drug sensitivity.

Introduction

In Dermatology department skin ulcer is common disease. It is tissue defects of the skin that extends

to the dermis and hypodermis, which can be induced by multiple causes. It is a clinically common disease that often affects patients' quality

of life and results in high medical costs. In addition, severe cases can threaten patient's life. Skin ulcers are frequently accompanied by bacterial infections, making the treatment procedure hard and outcome unsatisfactory. In particular, with the increasing use and misuse of antibiotics, the probability of infections by various opportunistic infections bacteria and drug-resistant bacteria has significantly increased, which further increases the difficulty of treatment. Skin ulcers compromise the skin's natural defense and lead to scar formation as well as poor blood perfusion, which further result in decreased local immunity. Moreover, the ulcer surface becomes an ideal colony for bacterial reproduction and invasion; as a result, mild cases may encounter difficulty in skin ulcer healing, and severe cases may need amputations or develop systematic infection and septicemia. These bad consequences significantly affect patients' physical health and their quality of life.^[1] Therefore, it is essential to culture ulcer surface bacteria, test drug sensitivity and analyze the bacterial infection pattern, through which we can monitor the distribution/variation of clinically common infectious bacteria and make appropriate selection of antibiotics. The most common bacteria cultured from these ulcer infections were *P. aeruginosa* and *S. aureus*, which is consistent with the findings reported by Renner et al.^[2] However, it is worth noting that, unlike previous studies^[3,4] showing that Gram positive bacteria, especially *S. aureus*, were the most common bacterial isolates in skin ulcer infections, Gram negative bacteria, especially *P. aeruginosa*, were more commonly isolated from the cases analyzed in the present study. Decreases in immunity resulting from various causes, including certain types of operations and invasive treatment procedures, make patients even more susceptible to bacterial infections.^[5]

Among the Gram-negative bacteria, the drug-resistance rate to commonly applied antibiotics, including ampicillin, ceftriaxone, cephazolin and piperacillin, was atypically high. All isolates of *P. aeruginosa* demonstrated the widest range of

antibiotic resistance to drugs including ampicillin, ampicillin/sulbactam, cephazolin, cefotetan, ceftriaxone, furantoin and SMZ/ TMP. Moreover, all the three isolates of *A. baumannii* were resistant to all tested drugs, which made it a super drug resistant bacterium. The antibiotics to which most Gram-negative bacteria were sensitive included amikacin, imipenem and piperacillin/tazobactam. Among the Gram-positive bacteria, the majority of isolates were resistant to most antibiotics, such as azithromycin, clarithromycin, clindamycin and erythromycin; however, the drug resistance of *S. epidermidis* was even greater. These results indicate that the infection bacteria at the ulcer surface have a strong resistance to the commonly used antibiotics. This situation may be related to the abuse of antibiotics in clinics, as well as the lag in research and development of antibiotics relative to bacterial evolution.^[4] The severe and multiple drug resistance of *P. aeruginosa* and *A. baumannii* were especially prominent; moreover, these organisms can easily form bacterial biofilm on ulcer surfaces, which significantly compromises the treatment effect. Infections by *P. aeruginosa* and *A. baumannii* have become a difficult problem in clinical settings.^[7,8] Therefore, in clinical practice, we should standardize the procedures to avoid the abuse of antibiotics and cross infection. We also need to improve bacterial culture and drug sensitivity testing to guide clinical medication and improve the therapeutic effect. At the same time, it can reduce the waste of medical resources and patient time and money.

To explore the constituent ratios and drug-resistance of the surface bacteria on skin ulcers, the present study analyzed 110 such cases hospitalized in the Dermatology Department of IMS and SUM Hospital, Bhubaneswar from Jan 2016 to Dec. 2017.

Materials and Methods

A total number of 110 patients with skin ulcers were enrolled in this study, including 74 male and 36 female, with the mean age of 52.7 years,

minimally 5 years and maximally 92 years. The shortest course of disease was 3 days and longest 34 years, mean 17 months. The causes included various wounds (burns, electric shock, scald, traffic accident wounds, scratch wounds, etc), surgical operations, diabetes, varicose veins and others. The involved areas included the feet (49 cases, 44.5%), the shank (45 cases, 40.9%), the torso (10 cases, 9%), the upper limbs (3 cases, 2.7%) and the head and face (3 cases, 2.7%). All the patients had received some treatment like dressing, debridement, folk recipe, etc before admission to our department, and 72 (65.5%) of them experienced external application of antiseptic drugs.

Bacterial culture on the day of admission to the hospital, for each patient, the secretion from the surface of the skin ulcer was collected using a sterile cotton swab, according to the established protocol, before any antibiotics have been administered. The samples were placed in sterile test tubes, sealed and immediately sent for testing. Pathogenic bacteria isolation and culturing were carried out based on the previous method.^[9,10]

Statistical Processing

SPSS 20.0 statistical processing software was used for statistical analysis. Data analysis adopted the descriptive method, and the intergroup comparison of constituent ratio employed with $p < 0.05$ indicating a statistically significant difference and $p < 0.01$ indicating a very significant difference.

Results

All wound swabs were cultured in microbiological culture medium. Among 110 samples 30 (27.3%) bacterial culture results were Gram-positive and 80 (72.7%) were Gram-negative. Altogether, 90 isolated bacteria were cultured; and the cultures from 10 patients demonstrated super infection with 2 types of bacteria. Constituent ratios of pathogenic bacteria Among the 90 identified bacterial isolates, 61 (67.8%) were Gram-negative bacteria, mainly comprising *Pseudomonas*

aeruginosa (26 isolates, 28.9%), *Klebsiella pneumoniae* (8 isolates, 8.9%), *Enterobacter cloacae* (6, 6.7%) and *Escherichia coli* (6, 6.7%). In addition, 23 isolates were Gram-positive bacteria (25.6%), mainly comprising *Staphylococcus aureus* (14 isolates, 15.6%) and *Enterococcus faecalis* (5 isolates, 5.6%). The constituent ratios of the bacterial cultures are documented (Table 1). Analysis of bacterial changes was performed for the two main types of infectious bacteria in the ulcer surface, *S. aureus* and *P. aeruginosa*, according to the number of positive specimens obtained from Jan. 2016 to Dec.2017. Results showed that the probability of a negative bacterial culture in 2017 was significantly lower than that in 2016 (16.7% vs. 40.0%, $p < 0.01$). Moreover, the probability of *P. aeruginosa* infection in 2017 was significantly higher than that in 2016 (31.7% vs. 14.0%, $p < 0.01$) (Table 2). Analysis of the drug sensitivity of ulcer surface bacteria Drug sensitivity of Gram-negative bacteria (Table 3) For *P. aeruginosa* (26 isolates), all (100.0%) of the isolates were resistant to ampicillin, ampicillin/sulbactam, cephalosporin, cefotetan, ceftriaxone, furantoin and sulfamethoxazole/trimethoprim (SMZ/ TMP); the vast majority (>90.0%) of the isolates were sensitive to amikacin, imipenem and piperacillin/tazobactam. For *K. pneumoniae* (8 isolates), <50.0% of the isolates were sensitive to piperacillin, ampicillin and furantoin; whereas all (100.0%) were sensitive to amikacin, ciprofloxacin, imipenem, piperacillin/tazobactam, SMZ/TMP and levofloxacin. For *E. cloacae* (6 isolates), <50.0% were sensitive to piperacillin/tazobactam and ampicillin/ sulbactam, whereas all (100.0%) were sensitive to amikacin, aztreonam, ceftazidime, ceftriaxone, imipenem and levofloxacin. For *E. coli* (6 isolates), <50.0% of the isolates were sensitive to gentamicin, tobramycin and ampicillin, whereas all (100.0%) were sensitive to amikacin, imipenem, ertapenem, cefotetan and Drug sensitivity of Gram-negative bacteria (Table 4, Fig 1a-d).

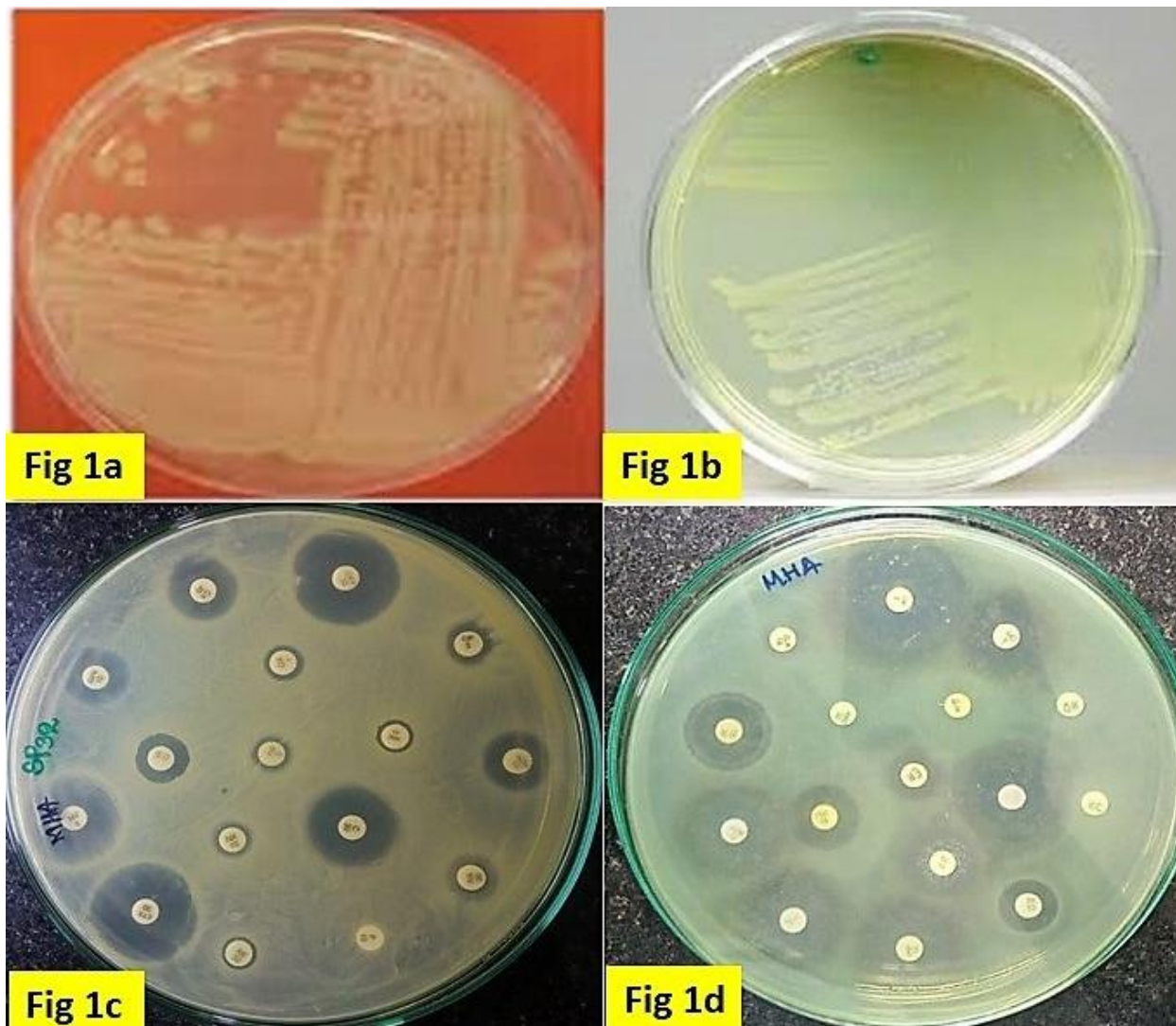


Fig 1a Pure culture of *Acinetobacter baumannii*, **Fig 1b.** Pure culture of *Pseudomonas aeruginosa*, **Fig 1c,d** Antibiotic sensitivity of isolated bacteria.

Table 1 Constituent ratios of pathogenic bacteria.

Pathogenic bacteria (n= 90)	No.	Percentage (%)
Gram-negative bacteria	61	67.8
<i>Pseudomonas aeruginosa</i>	26	28.9
<i>Klebsiella pneumoniae</i>	8	8.9
<i>Enterobacter cloacae</i>	6	6.7
<i>Escherichia coli</i>	6	6.7
<i>Acinetobacter baumannii</i>	3	3.3
<i>Serratia marcescens</i>	3	3.3
<i>Proteus Sp.</i>	3	3.3
<i>Morganella morganii Morgan subsp.</i>	2	2.2
<i>Klebsiella oxytoca</i>	1	1.1
Gram-positive bacteria	23	25.6
<i>Staphylococcus aureus</i>	14	15.6
<i>Enterococcus faecalis</i>	5	5.6
<i>Staphylococcus epidermidis</i>	3	3.3
<i>Staphylococcus haemolyticus</i>	1	1.1
Others (contaminating bacteria)	6	6.7

Note: Other are contaminating bacteria, such as coagulase negative staphylococcus, etc.

Table 2 Analysis of bacterial changes from 2011 to 2012 (No. of case).

Year	Bacterial species				Total
	No bacteria	<i>S. aureus</i>	<i>P. aeruginosa</i>	Others	
2011	20	6	7	24	50
2012	10	8	19	26	60
X ²	7.486	0.044	4.716	0.240	
p	0.006*	0.835	0.030*	0.030*	

*p< 0.05, compared with the two years.

Table 3 Analysis of the drug sensitivity of gram-negative bacteria at the ulcer surface.

Antibiotic	Bacteria species (sensitivity rate, %)				
	<i>p. aeruginosa</i>	<i>k. pneumoniae</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>A. baumannii</i>
Amikacin	96.2	100.0	100.0	100.0	0
Imipenem	96.2	100.0	100.0	100.0	0
Piperacillin/Tazobactam	92.3	100.0	33.3	100.0	0
Cefepime	88.5	87.5	83.3	83.3	0
ceftazidime	88.5	62.5	100.0	83.3	0
Levofloxacin	73.1	100.0	100.0	83.3	0
Tobramycin	76.9	87.5	16.7	33.3	0
Aztreonam	69.2	87.5	100.0	83.3	0
Ciprofloxacin	69.2	100.0	83.3	83.3	0
Gentamicin	65.4	75.0	50.0	50.0	0
Ampicillin	0	14.2	0	16.7	0
Ampicillin/sulbactam	0	87.5	33.3	83.3	0
Cephazolin	0	62.5	0	66.7	0
Cefotetan	0	87.5	16.7	100.0	0
Ceftriaxone	0	75.0	100.0	83.3	0
Furantoin	0	12.5	16.0	83.3	0
Sulfamethoxazole/Trimethoprim	0	100.0	0	66.7	0
Cefuroxime	-	87.5	-	-	-
Meropenem	-	87.5	-	-	0
Piperacillin	-	25.0	-	-	-
ertapenem	-	-	83.3	100.0	0

Table 4 Analysis of the drug-sensitivity results for gram-positive bacteria at the ulcer surface.

Antibiotics	Bacteria species (Sensitivity rate, %)		
	<i>S. aureus</i>	<i>E. faecalis</i>	<i>S. epidermidis</i>
Linezolid	100.0	-	100.0
Moxifloxacin	100.0	80.0	66.7
Furantoin	100.0	-	100.0
Quinupristin/Dalfopristin	100.0	0	100.0
Tigemycin	100.0	100.0	100.0
Vancomycin	100.0	100.0	-
Rifampin	92.3	-	100.0
Levofloxacin	85.7	50.0	33.3
Oxacillin	85.7	50.0	33.3
Cefaclor	85.7	-	33.3
Cefotaxime	78.5	-	33.3
Ceftriaxone	78.5	-	66.7
cefuroxime	78.5	-	33.3
Ciprofloxacin	78.5	60.0	33.3
Gentamycin	64.3	-	33.3
SMZ/TMP	64.3	0	33.3
Tetracyclin	57.1	0	100.0
Azithromycin	14.3	-	0
Clarithromycin	14.3	-	0
Clindamycin	14.3	0	33.3
Erythromycin	14.3	0	0
Penicillin G	14.3	100.0	33.3

For *S. aureus* (14 isolates), <50.0% of the isolates were sensitive to azithromycin, clarithromycin, clindamycin, erythromycin and penicillin G, whereas all (100.0%) of the isolates were sensitive to linezolid, moxifloxacin, furantoin, quinupristin/dalfopristin, tigemycin and vancomycin. For *Enterococcus faecalis* (5 isolates), <50.0% were sensitive to quinupristin/dalfopristin, SMZ/TMP, tetracycline, clindamycin and erythromycin, whereas all (100.0%) of the isolates were sensitive to tigemycin, vancomycin and penicillin G. For *Staphylococcus epidermidis* (3 isolates), <50.0% were sensitive to levofloxacin, oxacillin, cefaclor, cefotaxime, cefuroxime, ciprofloxacin, gentamicin, SMZ/TMP, azithromycin, clarithromycin, clindamycin, erythromycin and penicillin G, whereas all (100.0%) of the isolates were sensitive to linezolid, furantoin, quinupristin/dalfopristin, tigemycin, rifampin and tetracycline.

Discussion

Skin ulcers compromise the skin's natural defense and lead to scar formation as well as poor blood perfusion, which further result in decreased local immunity. Moreover, the ulcer surface becomes an ideal colony for bacterial reproduction and invasion; as a result, mild cases may encounter difficulty in skin ulcer healing, and severe cases may need amputations or develop systematic infection and septicemia. These bad consequences significantly affect patients' physical health and their quality of life.^[9] According to one study, 137 study subjects, bacterial pathogens were isolated from 115 patients with the isolation rate of 83.9%. This was higher than the previous study done in Gondar (52%), Bahir Dar (53%), Dessie (70.5%), and Addis Ababa (42%), Ethiopia^[9,10,11,12]. This high rate of bacterial isolation in the present study may be due to the differences of the quality of wound swab specimens and bacteriological techniques (overnight incubation in BHI) used. On the other hand, the type of wound pathogens and their rate of isolation in these findings were found to be consistent with study conducted in India

(79%)^[13]. However, it was lower than a study done in Nigeria (94%)^[14].

This finding is consistent with a retrospective study done in Gondar^[9]. The reason may be chronic wounds tending to show monomicrobial infections. The present study revealed polymicrobial infections, mainly by *Klebsiella spp.* and *S. aureus*, which is consistent with report from India^[13]. The current findings showed that the rates of isolation of Gram-negative and Gram-positive were 56.6% and 43.4%, respectively. This was in agreement with studies done in Zaria, Nigeria, 55% and 44%, respectively^[15]. However, the present result is different from the previous report from Gondar University Hospital, Ethiopia (29% versus 71%)^[9]. The present findings show higher rates of isolation of Gram-negative wound pathogens from the same area. This high rate of Gram-negative and low rate of Gram-positive isolates from wound in the same area may be due to high number of cases included from inpatients in the present study compared to outpatients. This may probably contribute high number of Gram-negatives than Gram-positives.

The predominant isolate in the present study was found to be *S. aureus*, which was 34%; this finding was higher when compared with previous reports from Italy (28.2%)^[16] and Nigeria (25.1% and 25%)^[17,18]. This difference may be due to improved facilities of the hospital management from these countries in the infection prevention and control program. However, it is lower than reports in Nigeria (44%) and other parts of Ethiopia Dessie (41.6%), Bahir Dar (69.7%), and Gondar (65.5%)^[9,10,12,15]. The second predominant Gram-negative bacterium in this study was *Klebsiella spp.* 17 (12.5%). Similar result was reported from Cape, South Africa, which revealed that *K. pneumoniae* were the second predominant organisms isolated (13.4%)^[19].

In this study *Cons* accounted for 11.8% of the isolates. This finding is similar to a report in Nigeria, where *S. epidermidis* accounted for 11.4%^[18]. The percentage of *Citrobacter spp.* 15 (11%) is higher than the previous studies in

Gondar University Teaching Hospital 1 (1.3%), Bahr Dar 2 (0.9%), and Dessie Ethiopia 21 (4.2%) [9,10,12]. Among the 15 isolates of *Citrobacter spp.* 100% showed MDR and this alarms that multiple drug resistant strains of *Citrobacter spp.* circulate in the study area. In the present study the susceptibility pattern of *S. aureus* isolates demonstrated high level of resistance to the commonly used antimicrobial agents. This result is in agreement with a study done in Jimma^[20]. The present study showed a single isolate vancomycin-resistant Enterococcus spp. (VRE), indicating the emergence of VRE may pose therapeutic problems. Oxacillin-resistant *Cons* has become the predominant pathogen. According to the current study oxacillin-resistant *Cons* were 12 (75%); this is in line with a study reported in Nigeria (77.3%)^[21]. The percentage of isolates that were resistant to cloxacillin was 38.9% which was similar to a study done in Addis Ababa, Ethiopia (37.2%)^[11]. Showing that, they may be reservoirs for methicillin-resistant *Staphylococcus aureus*, as they are common nosocomial wound infections. However, this finding was inconsistent with a report in Nigeria (98.3%)^[18]. Ciprofloxacin was relatively sensitive for both Gram-positive and Gram-negative isolates except *Klebsiella spp.* However, level of resistance to ciprofloxacin is increasing from 16% in 2006^[9] to 36% in the present study in the same study area. The present study demonstrated that amoxicillin was resistant to 83% of Gram-positives which was higher than a study done in Dessie reported as amoxicillin had the highest resistance rate 78.9%^[12]. This sharp increase resistance patterns may be due to overuse of it as empiric treatment option for most of the patients. The current finding also showed that *S. aureus* isolated from inpatients was more resistant than from outpatients. Similarly, a study done in Jimma reported that inpatient isolates of *S. aureus* were more resistant than outpatient isolates to all the tested antibiotics except erythromycin^[20].

Overall MDR patterns of the isolated pathogens were 130 (95.5%), this is in line with the studies

conducted in Bahir Dar, Ethiopia, 95.5% [10] and higher than previous study in 2006 which was 78.5% [9]. This may be due to massive use of antimicrobials in the area without prescription and as empirical treatment option by physicians or prolonged use of antibiotics may be responsible for the development of more resistant strains of the pathogens.

Conclusions

From this study it is concluded that the empirical treatment should be stopped. Before prescribing any antibiotics the drug sensitivity should be carried out. As conventional methods are time consuming the early detection method i.e PCR should be implemented.

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