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BACTERIAL PATHOGENS OF NOSOCOMIAL INFECTIONS IN ICUS AND THEIR ANTIBIOTICS RESISTANT PATTERN AT KING KHALID HOSPITAL IN AL-KHARJ/SAUDI ARABIA

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Abstract:

Background:*The intensive care unit [ICU] is considered as infection epicenter because vulnerable population of critically ill patients and use of different invasive devices. Consequently, the ICU population has one of the highest occurrence rates of nosocomial infections leading to an enormous impact on morbidity, hospital costs, and often survival. In addition, the increasing problem of antibiotic resistance loads the burden of nosocomial infection in the ICU. Constant and careful global monitoring for multidrug-resistant bacteria is needed to minimise the possibility of appearance and dissemination of new resistant isolates and to avoid complications in treatment choices.*

Methods:*This study was carried out from March to June 2016 in King Khalid Hospital [Al-Kharj-KSA] to explore the multidrug-resistant bacteria, Extended Spectrum β- lactamase bacteria [ESBLs] and the possibility of carbapenems resistant bacteria isolated from clinical samples of patients in the ICUs. A total of 317 different clinical samples were received for cultivation and antibiogram during the study period. Samples were cultivated on Blood agar, MacConkey agar, CLED, EMB agar and Mannitol salt agar. Gram stain, colony morphology and biochemical tests were done.The final identification results of the causative agents and its sensitivity profile were obtained by automated procedures "Phoenix 100/BD company". Minimum inhibitory concentration [MIC] results were interpreted according to Clinical and laboratory standard institute [CLSI] guidelines.*

Results:*Out of 317 total samples processed during the study, significant growth was shown in 62 samples [19.5%]. Respiratory samples showed the highest rate of positive growth [40.3% out of 62] followed by urine [20.96% out of 62]. Fifty-seven isolates [91.94 %] were gram-negative and five isolates [8.06%] were gram-positive.*

K. pneumoniae was the most frequently isolated among Gram-negative with16 isolates [28%] followed by P. aeruginosa 12 [21%].

All isolates of P. aeruginosa, Acinetobacter spp., Providencia spp., Enterobacter spp., Citrobacter spp., Serratia spp. were MDR [100%] while five isolates [71.4%] of Proteus mirabilis, and 11 [69%] of K. pneumoniae were MDR. ESBLs were confirmed in 39 [83%] isolates out of 47 MDR gram-negatives; among them, 11[28.2%] were K. pneumoniae and10 [25.64%] isolates of P. aeruginosa. Resistance to carbapenems was detected in 23 [48.94%] isolates of MDR gram-negative bacteria; among them, 10 [43.48%] isolates of P. aeruginosa, and 6[26.1%] isolates each of Acinetobacter spp. and K. pneumoniae.

Conclusion:*Considerable efforts and regular evaluation of ESBL and carbapenems resistant bacteria are of great importance both in hospital and community to avoid the appearance of new bacterial isolates which may resist all clinically used antibiotics.*

Keywords: *Nosocomial infection, MDR, ESBL, Carbapenems, Resistance, Bacteria.*

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INTRODUCTION:

Nosocomial infection defined as a condition that results from an adverse reaction to the presence of an infectious agent or its toxins after 48 hours of admission to the hospital [1, 2]. It has estimated that 90,000 deaths per year worldwide are due to nosocomial infection [2-5, 24]. In the developed countries, it has reported that from 5% to 15% of hospitalised patients become infected in regular wards and as many as 50% or more of patients in intensive care units [ICUs] [6-9].

Recent treatments command the use of intravenous/ urinary catheters, respirators, hemodialysis, complicated operations, therapy using cortisone and others which depress defence mechanisms and make patients susceptible to infections such as urinary tract infection, pneumonia, surgical infection, catheter infection, bacteremia, and other infections [3,12,13,19, 21, 22].

The most common bacteria associated with ICU infections are *E. coli, Pseudomonas aeruginosa*, *Acinetobacter* spp., *S. aureus*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Citrobacter* sp. and others [10,11,14-17].

The sources of these organisms may be the patient's flora, visitors, ICU environments like water, air, foods, and equipment, health care workers, other patients, or inanimate objects that are in close to patients [12,13,18, 20].

Bacterial resistance is a serious problem in the hospital environment, especially when the infection is caused by the multidrug resistance organism [23]. Several different mechanisms of bacterial drug resistance have been described, for example, production of various drug-inactivating enzymes like β- lactamases, multiple efflux pump, and reduced uptake [25].

This study was aimed to explore the multidrug-resistant bacteria, Extended Spectrum β- lactamase bacteria [ESBLs] and the possibility of carbapenems resistant bacteria isolated from clinical samples of patients in the ICUs [adult, pediatric and neonatal ICU].

METHODS:

This study was carried out from March to June 2016 in King Khalid hospital in Al-Kharj after getting ethical approval from King Fahad Medical City/Riyadh. IRB No. 16-010E.

Samples

A total of 317 different samples [Urine, wound, Blood, Respiratory, and others] were received by Microbiology Lab from ICUs for cultivation and antibiogram during the study period [Table 1]. There was no direct contact with patients, and there was no usage of any antibiotics for patients in the research project.

Isolation of bacteria

All clinical specimens received by Microbiology lab were treated according to good laboratory practice and standard methods for identification.

Urine and tracheal aspirates, a loop full was inoculated onto Blood agar [BA] and MacConkey agar [MA], CLED, EMB agar and Mannitol salt agar and aerobically incubated at 37^0 C for 24 hours. Pus and wound swabs were inoculated onto BA, MA, EMB, Chocolate agar [CA] and Mannitol salt agar [MSA].

The BA and CA plates were incubated at37^0 C for 24 hours at 5–10% CO2 whereas MA, EMB and MSA were incubated aerobically at 37^0 C for 24 hours.

Blood samples were collected in Bactec blood culture bottles [BD Blood Culture System, Becton] and incubated at 37°C in Bactec 9240 following manufacturer instructions. Positive bottles were subcultured on BA, CA, MSA, EMB agar and MAC agar.

Gram stain, colony morphology and biochemical tests [catalase, oxidase, coagulase] were done for initial screening. The final identification results of the causative agents were confirmed by automated procedures "Phoenix 100/BD company".

Antibiotic susceptibility

Phoenix 100/BD company machine is used in Microbiology lab/ King Khalid hospital for identification of bacteria from clinical samples and antibiogram. The antibiotics used for testing Gram - negative and Gram- positive are shown in Table 2, and 3, and the minimum inhibitory concentration [MIC] results were interpreted according to Clinical and laboratory standard institute [CLSI] guidelines [26].

Multidrug Resistance

Multidrug-resistant bacteria [MDR] isolates were defined when the results show the bacteria as resistant to three or more antibiotics belonging to different structural classes.

Extended-spectrum β-lactamase [ESBLs] gram-negative bacteria

ESBLs were defined as the bacteria which hydrolyze and cause resistance to β-lactam antibiotics including the third generation of cephalosporins [Ceftazidime, Ceftriaxone] and monobactams [aztreonam] but not carbapenems.

Resistance to Carbapenems

The isolates which are ESBLs and show resistance to one or more of carbapenems used [Imipenem, Meropenem, Ertapenem] were identified as possibly carbapenemase producers [51].

RESULTS:

Out of 317 total samples processed during the study, 60 samples [18.93%] showed significant

growth. Respiratory samples 25 [41.66%] were the most frequent positive samples followed by urine 13[21.66%] blood and wound 6 each [10%] and other samples including eye swabs, ear swabs and umbilical swabs 10 [16.66%] Figure 1&Table 1.

Out of 60 total isolates, 57 [95%] were Gram-negatives, and 3 [5%] were gram-positive. *K. pneumoniae* was the most frequently isolated among Gram-negatives with 16 isolates [26.66%] followed by *P. aeruginosa* 12 [20%]. Gram-positive isolates were *Staphylococcus aureus* and MRSA. All results are given in Figure 1&Table 1.

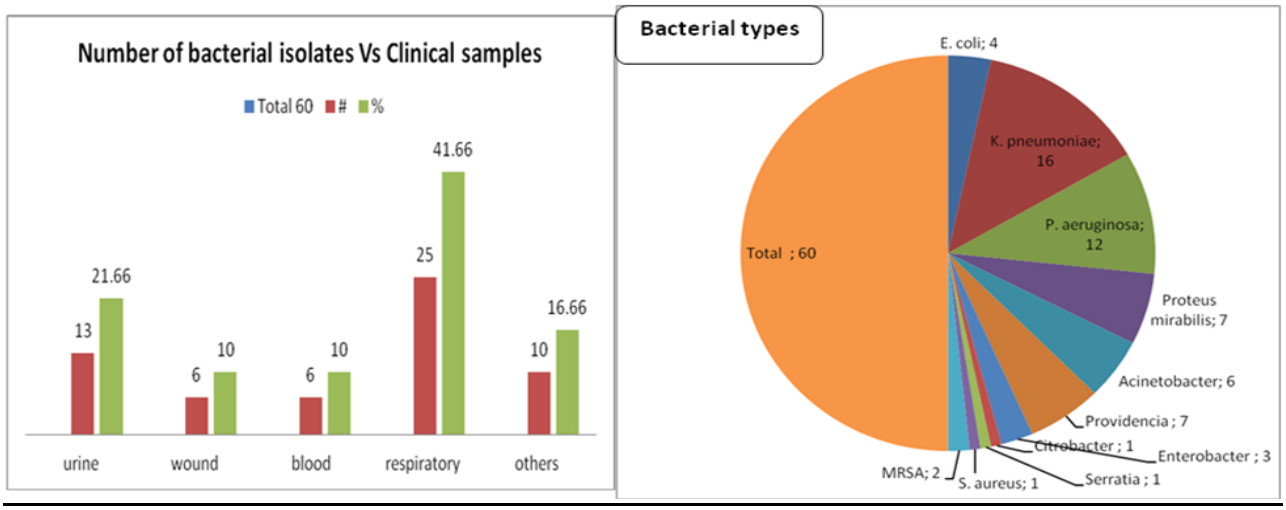

Fig 1: Right: The bacterial types isolated. Left: Total number of bacteria isolated from clinical sample.

Table 1: The number of bacterial types isolated Vs clinical samples.

Bacteria	**No. of isolates**	**Samples**				
		Urine	**Wound**	**Blood**	**Respiratory**	**Others**
E. coli	**4**[6.66%]	1	1	0	0	2
K. pneumoniae	**16**[26.66%]	4	0	3	4	5
P. aeruginosa	**12**[20%]	1	0	0	11	0
Proteus mirabilis	**7**[11.66%]	1	2	1	3	0
Acinetobacter	**6**[10%]	1	1	0	2	2
Providencia	**7**[11.66%]	3	2	1	0	1
Enterobacter	**3**[5%]	2	0	0	1	0
Citrobacter	**1**[1.66%]	0	0	0	1	0
Serratia	**1**[1.66%]	0	0	0	1	0
S. aureus	**1**[1.66%]	0	0	1	0	0
MRSA	**2**[3.33%]	0	0	0	2	0
Total	**60 [100%]**	**13** [21.66%]	**6** [10%]	**6** [10%]	**25** [41.66%]	**10** [16.66%]

High resistant rates of *K. pneumoniae* was noticed against antibiotics like ampicillin [100%], each of cephalothin, cefuroxime, ceftriaxone [69%], ceftazidime and Amox/Calv [62%] and cefepime, aztreonam, nitrofurantoin each [56%]. *K. pneumoniae* showed high sensitivity to meropenem [100%].

Similarly, high resistance to each of imipenem, meropenem and aztreonam [83%] followed by ceftazidime and ciprofloxacin [75%] was found against *P. aeruginosa* but it was highly sensitive to each of amikacin and gentamicin [100%]. Detailed results are given in Table 2.

All isolates of *P. aeruginosa, Acinetobacter spp., Providencia spp., Enterobacter spp., Citrobacter spp., Serratia spp.* were MDR [100%] while five isolates [71.4%] of *Proteus mirabilis*, 11 [69%] of *K. pneumoniae* and one isolate [25%] of *E. coli* were MDR. Detailed results are shown in Table 4.

Among Gram-positive bacteria, *Staphylococcus aureus* [one isolate] and MRSA [2 isolates] were identified. *Staphylococcus aureus* shows high sensitivity to mostly all of the antibiotics used while both MRSA isolates were MDR but show high sensitivity to many antibiotics such as vancomycin, nitrofurantoin, daptomycin and teicoplanin [100%]. Results are given in Table 3&4.

According to the CLSI definition, ESBLs were confirmed in 39 [83%] out of 47 MDR gram-negative isolates. Among them, 11 [28.2%] were *K. pneumoniae*, 10 [25.64%] isolates of *P. aeruginosa*, 7 [17.95%] *Providencia* spp., *Acinetobacter* spp. 6 [15.4%], *Proteus mirabilis* 3[7.7%] and only one isolate of *Serratia* spp. and *E. coli* [2.56%].

The possibility of resistance to carbapenems was observed in 23 [48.94%] isolates of MDR gram-negative bacteria; among them 10 [43.48%] isolates of *P. aeruginosa*, 6 [26.1%] each of *Acinetobacter* spp. and *K. pneumoniae* and one isolate only of *Serratia* spp. Detailed results are presented in Figure 2 &Table 4.

Table 2: Gram-negative bacteria and its sensitivity profile with 20 different antibiotics.

Bacteria	#	AK	GN	ERT	IMI	MEM	KF	CXM	FOX	CAZ	CRO
E. coli	4	100	100	100	100	100	25	75	100	75	75
K. pneumoniae	16	81	63	75	88	100	31	31	69	38	31
P. aeruginosa	12	100	100	0	17	17	0	0	0	25	0
Proteus mirabilis	7	86	86	71	-	57	43	71	86	71	57
Acinetobacter	6	0	0	0	-	0	0	0	0	0	0
Providencia	7	100	0	100	-	100	0	0	100	0	0
Enterobacter	3	100	100	100	100	100	67	67	67	100	100
Citrobacter	1	100	100	100	100	100	100	100	100	100	100
Serratia	1	100	100	0	0	100	0	0	0	100	0

Cont.

Bacteria	#	CPM	ATM	AMP	AUG	PRL/TAZ	TS	NI	CIP	LEV	TIG
E. coli	4	75	75	25	75	100	0	100	50	50	100
K. pneumoniae	16	44	44	0	38	63	50	44	50	75	88
P. aeruginosa	12	25	17	0	0	58	0	0	58	42	-
P. mirabilis	7	57	57	29	29	100	14	0	57	71	0
Acinetobacter	6	0	0	0	0	0	67	0	0	0	0
Providencia	7	0	0	0	0	100	0	0	0	0	0
Enterobacter	3	100	100	0	67	100	100	100	100	100	67
Citrobacter	1	100	100	0	100	100	100	100	100	100	100
Serratia	1	0	100	0	0	100	100	0	100	100	0

AK:Amikacin, GN: Gentamicin, ERT: Ertapenem, IMI: Imipenem, MEM: Meropenem, KF: Cephalothin, CXM: Cefuroxime, FOX: Cefoxitin, CAZ: Ceftazidime, CRO: Ceftriaxone, CPM: Cefepime, ATM: Aztreonam, AMP: Ampicillin, AUG: Amox/Calv, PRL/TAZ: Piperacillin/Tazobactam, TS: Trimethoprim/Sulfa, NI: Nitrofurantoin, CIP: Ciprofloxacin, LEV: Levofloxacin, TIG: Tigecycline.

Table 3: Gram-positive bacteria and its sensitivity profile with 21 different antibiotics.

Bacteria	#	GN	IMI	FOX	CTX	AMP	PG	OX	AUG	DAP	TS	TEIC
S. aureus	1	100	100	-	100	0	0	100	100	100	100	100
MRSA	2	50	0	0	0	0	0	0	0	100	100	100
Enterococcus	2	0	-	0	0	50	0	-	-	100	0	100

Bacteria	#	VAN	CD	E	LIN	MU	NI	CIP	MOX	RIF	TC
S. aureus	1	100	100	100	100	100	100	100	100	100	100
MRSA	2	100	100	100	100	100	100	100	100	100	0
Enterococcus	2	100	0	0	0	-	100	0	0	-	50

GN: Gentamicin, IMI: Imipenem, FOX: Cefoxitin, CTX**:** Cefotaxime. AMP: Ampicillin, PG: PencillinG. OX: Oxacillin. AUG: Amox/Calv, DAP: Daptomicin. TS: Trimethoprim/Sulfa. TEIC: Teicoplanin. VAN: Vancomycin, CD: Clindamycin. E: Erythromycin. LIN: Linezolid. MU: Mupirocin high level. NI: Nitrofurantoin, CIP: Ciprofloxacin, MOX: Moxifloxacin.RIF: Rifampin.TC: Tetracycline.

Table 4: Multiple drug resistant, ESBLs and carbapenems resistant isolates of gram positive and gram negative .

Bacteria	**No. of isolates**	**No. of MDR/%**	**No. ESBLs/ % Of MDR**	**No. Resistant Carbapenems/% of MDR**
E. coli	**4**	**1**[25%]	**1**[100%]	**0** [0%]
K. pneumoniae	**16**	**11**[69%]	**11**[100%]	**6** [37.5%]
P. aeruginosa	**12**	**12**[100%]	**10**[83.33%]	**10** [83.33%]
Proteus mirabilis	**7**	**5** [71.4%]	**3**[60%]	**0** [0%]
Acinetobacter	**6**	**6** [100%]	**6**[100%]	**6** [100%]
Providencia	**7**	**7**[100%]	**7**[100%]	**0** [0%]
Enterobacter	**3**	**3**[100%]	**0**[0%]	**0** [0%]
Citrobacter	**1**	**1**[100%]	**0**[0%]	**0** [0%]
Serratia	**1**	**1**[100%]	**1**[100%]	**1** [100%]
S. aureus	**1**	**0**[0%]	-	-
MRSA	**2**	**2**[100%]	-	-
Total	**60**	**49/60** [81.66%]	**39/47** [83%]	**23/47** [49%]

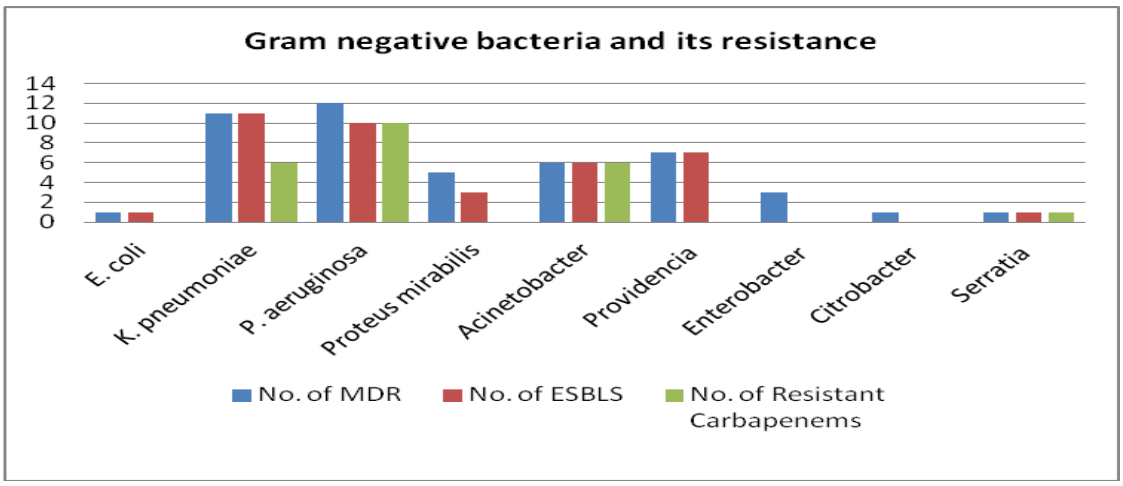

Fig 2: Multiple drug resistant, ESBLs and carbapenems resistant isolates of gram negative bacteria.

DISCUSSION:

The development of antimicrobial resistance started as soon as the antibiotics were used clinically in 1940. Methicillin-resistant *S. aureus* [MRSA] had been evolved worldwide in 1961which forced the use of vancomycin in chronically and severely ill patients resulting in the rise of MRSA with reduced susceptibility to vancomycin [27-30]. The continuing exposure of bacterial strains to some β-lactams has provoked persistent production and mutation of β-lactamases among gram-negative bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* . Such enzymes are known as Extended-spectrum β-lactamases [ESBLs] which cause resistance to βlactams including the third generation of cephalosporins [cefotaxime, ceftriaxone, ceftazidime] and monobactams [aztreonam] but not carbapenems [31,32, 33].

In this study low growth rate was found from different clinical samples compared with the results have been reported in the previous studies carried out in ICUs [34,35,36]. The commonest sites of infection were respiratory tract infections followed by urinary tract and bloodstream infections, and gram-negative bacteria such as *K. pneumoniae* and *P. aeruginosa* were the most prevalent pathogens isolated from ICU patients in this study.

These findings are compatible with other studies [36,38,39,41]. However, in other studies, it has been shown that *Acinetobacter* spp. are the major nosocomial pathogens of ICU [35,37,40]. This difference may be attributed to the difference in geographical location, nutritional status, health care settings, and immune status of the patient.

In this study, all isolates of *P. aeruginosa* , *Acinetobacter* spp., *Providencia* spp., *Enterobacter* spp., *Citobacter* spp., *Serratia* spp. and isolates of Gram-positive were MDR while *Proteus mirabilis* [71.4%] *K. pneumoniae* [69%] and *E. coli* [25%] were MDR which almost shows similar result reported in earlier studies [34,35,42].

Out of 39 ESPL isolated, the higher prevalence was found in *K. pneumoniae* 11[28.2%] isolates followed by10 [25.64%] isolates of *P. aeruginosa*, 7 [17.95%] *Providencia* spp., and *Acinetobacter* spp. 6 [15.4%].

A previous study in Nepal reports that a prevalence rate of 28.6% of *K. pneumoniae* isolates [35, 43] and a study in Saudi Arabia conclude that 26% of *K. pneumoniae* were ESBLs [44]. Moreover, data over three years investigation in Kuwait showed that the levels of ESBLs of *K. pneumoniae* and *E. coli* isolated from urine samples of inpatient were 28% and 26%, respectively [45]. A recent study in a tertiary hospital in Patiala, Punjab showed that ESBL production was confirmed in 50% of *P. aeruginosa*, 48% of *E. coli*, and 44% of *K. pneumoniae* isolates [46]. A study carried out by Majda *et al*. reported that 72% of *E. coli* and 65.8% of *K. pneumoniae* isolated from urine samples were ESBL producers [47]. In a study done by Shakti *et al*. reported that ESBL positive among ICUs isolates was 12.11%, and ESBL positive from nosocomial isolates was 22.47% [48].

The ESBL rate differs between countries due to the difference in the geographical area, the hospital, the community, the host and the bacteria and their mobile genetic elements.

Moreover, several risk factors exist for infection with ESBL producer like chronic ill patients with an extended stay in the hospital, use of invasive devices, extensive antibiotic use, recent surgery, gastrostomy, and hemodialysis [12, 13, 19, 24].

For a long time, carbapenems [imipenem, meropenem] are considered as the first choice for the treatment of many infections caused by ESBLs producing bacteria, but unfortunately carbapenemase resistant isolates have been evolved in the past years in many countries [10, 49,50, 51]. E.G. Playford *et al*. conclude that 4.6% of patients admitted to ICU for more than 48 hours acquired carbapenem-resistant *Acinetobacter baumannii*[10]. A study carried out in 7 US Communities, Guh AY. *et al*. reports that the overall annual Carbapenem-Resistant Enterobacteriaceae incidence rate per 100000 population was 2.93 which were isolated mostly from urine and blood [51]. In this study, 23 [48.94%] out of 47 MDR gram-negative isolates in which all *Acinetobacter* spp. 6 [100%] isolates, *P. aeruginosa* 10 [83.33%] isolates and *K. pneumoniae* 6 [54.55%] were potential carbapenems resistant.

CONCLUSION:

The frequency of infections caused by ESBL and carbapenems resistant bacteria has increased in recent years. Detection of ESBL and MDR carbapenems is of great importance both in hospital and community. The prevalence and incidence of these bacteria are becoming more complicated with increasingly fuzzy borders between community and hospitals.

Probably, a ‘‘super germ’’, resistant to relatively all clinically used antibiotics, is expected in the future. Constant and careful worldwide monitoring for multidrug-resistant bacteria is urgently warranted.

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