ORIGINAL ARTICLE

The Impact of Aerators on Water Contamination by Emerging Gram-Negative Opportunists in At-Risk Hospital Departments

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(See the commentary by Decker and Palmore, on pages 130-131.)

OBJECTIVE. Our aim was to evaluate the impact of aerators on water microbiological contamination in at-risk hospital departments, with a view to quantifying the possible risk of patient exposure to waterborne microorganisms.

DESIGN. We analyzed the microbiological and chemical-physical characteristics of hot and cold water in some critical hospital departments. SETTING. Two hospitals in northern Italy.

METHODS. We took 304 water samples over a 1-year period, at 3-month intervals, from taps used by healthcare personnel for handwashing, surgical washing, and the washing of medical equipment. We analyzed heterotrophic plate counts (HPCs) at 36°C and 22°C, nonfastidious gram-negative bacteria (GNB-NE), and Legionella pneumophila.

RESULTS. The percentages of positivity and mean values of HPCs at 22°C, HPCs at 36°C, and GNB-NE loads were significantly higher at outlet points than in the plumbing system. In particular, GNB-NE positivity was higher at outlet points than in the plumbing system in both the cold water (31.58% vs 6.58% of samples were positive) and hot water (21.05% vs 3.95%) supplies. Our results also revealed contamination by *L. pneumophila* both in the plumbing system and at outlet points, with percentages of positive samples varying according to the serogroup examined (serogroups 1 and 2–14). The mean concentrations displayed statistically significant (P < .001) differences between the outlet points (27,382.89 \pm 42,245.33 colony-forming units [cfu]/L) and the plumbing system (19,461.84 \pm 29,982.11 cfu/L).

CONCLUSIONS. These results reveal a high level of contamination of aerators by various species of gram-negative opportunists that are potentially very dangerous for immunocompromised patients and, therefore, the need to improve the management of these devices.

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The water distribution system in hospitals may constitute a source of healthcare-associated infections (HAIs) caused by opportunistic pathogens such as *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, *Acinetobacter* species, and fungi. ¹⁻⁶ These organisms are transmitted by direct contact (eg, hydrotherapy, bathing, and debridement), ingestion of water, indirect contact (eg, improperly reprocessed medical devices), inhalation of aerosols generated by a water source, and aspiration of contaminated water. ^{7,8} In particular, *P. aeruginosa* can persist in hospital water for long periods ⁹ and can cause nosocomial outbreaks of disease, ¹⁰ frequently involving drug-resistant organisms. ⁷

Immunocompromised patients are particularly susceptible to infection by such microorganisms, which can cause bacteremia, pneumopathy, meningitis, and other conditions. One of the emerging microorganisms most frequently involved in these pathologies is *P. aeruginosa*, which is reported to be lethal in 50%, 70%, and 20% of bacteremia, pneumopathy, and meningitis cases, respectively.

The quality of drinking water is subject to many regulations based on lifetime health effects in the general population. However, with regard to people with increased susceptibility to infection, insufficiently broad water quality indicators are used (eg, they do not include opportunistic pathogens), and there is a lack of guidelines covering all the various healthcare

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settings.7,14 Only a few European countries (United Kingdom, France, and Germany) and the US Centers for Disease Control and Prevention (CDC) have drawn up guidelines for water quality in healthcare facilities.8,15 The CDC recommendations include strategies to minimize the growth and persistence of gram-negative waterborne bacteria, such as the recommendation that cold water in healthcare facilities should be stored and distributed at temperatures below 20°C and that hot water should be stored above 60°C and circulated with a minimum return temperature of 51°C.

In Germany and France, environmental surveillance of water systems is an integral part of the infection control programs used. 16 The aim of our study was to evaluate the microbiological quality of the water supply in a few critical hospital departments, with a view to quantifying the possible risk of exposure of patients to waterborne opportunistic gram-negative microorganisms and to determining whether this risk is engendered by contamination attributable to the plumbing system or to the use of aerators, an aspect that, to our knowledge, has not yet been investigated.

METHODS

During 2012, we analyzed the microbiological and chemicalphysical characteristics of the water supply in a few critical departments (intensive care unit [ICU], operating suite, and neonatology unit) in 2 tertiary care hospitals in northern Italy that were composed of separate pavilions with a total of 491 and 430 beds. In the hospitals considered, no routine procedure is implemented for the prevention of waterborne infections caused by emerging gram-negative opportunists, except for Legionella species, for which Italian legislation makes provision for specific measures of prevention and control.

Water Distribution System

The water entering the hospitals is supplied exclusively by the public water authority, and the hospitals do not implement any additional disinfection treatments of the drinking water from the main supply.

Water Sampling

In all of the critical departments considered, we sampled water from those taps that were not equipped with point-ofuse filters but were nevertheless regularly used. A total of 19 taps were examined, and samples of hot and cold water were taken from each.

Water sampling was performed both in conditions of "ordinary use" (to assess the actual risk at each outlet point) and after running the water for 2 minutes (to analyze the microbiological features of the plumbing system). Specifically, samples of both hot and cold water were first taken while the aerator of the tap was in place (ie, in conditions of normal use). Subsequently, the outlet point was disinfected and flame-sterilized, and the water was run. A total of 304 water samples were taken over a 1-year period at 3-month intervals

(16 samples for each of 19 taps). The samples were taken from taps used by healthcare personnel for handwashing, surgical washing, and the washing of medical equipment. In addition, the supply of water entering the hospital was tested to evaluate its overall quality at the source.

We analyzed the following microbiological characteristics of both the hot and cold water circuits: heterotrophic plate counts (HPCs) at 36°C and 22°C and nonfastidious gramnegative bacteria (GNB-NE). With regard to the hot water system, we also evaluated the concentration of L. pneumophila and its serogroups and of other species of Legionella. At the same time, temperature and residual free chlorine content were measured (Visocolor HE; Machereye Nagel).

Processing of Samples

Samples of tap water were collected by means of the pre- and post-flushing modality. For the sampling of microbiological parameters, sterile plastic bottles were used and sodium thiosulphate solution was added to the samples to neutralize free chlorine. The samples were immediately transported in portable coolers (at 4°C) to the laboratory for chemical and microbiological analysis and processed within 24 hours.

Determination of HPCs at 36°C and 22°C

HPCs at 36°C and 22°C were determined in duplicate by means of the pour plate method using ISO 622217 conform yeast extract agar (Merck), and 1 mL of sample was pipetted onto petri plates. Approximately 15 mL of molten yeast extract agar medium was dispensed onto each petri plate and gently swirled several times to mix the water sample in the growth medium. The agar was left to solidify and, after complete solidification of the medium, the inoculated plates were inverted and incubated at 36°C for at least 48 h and at 22°C for at least 72 h. The total number of colonies was reported in colony-forming units (cfu) per milliliter.

Isolation and Identification of GNB-NE

To recover GNB-NE, 100-mL samples of water were filtered through 0.45-µm cellulose filters (Millipore) and placed on tryptic soya agar medium (bioMérieux). Inoculated agar plates were incubated for 24 h at 36°C. The grown colonies were counted and differentiated, and the isolates were identified at the species or genus level by means of the API Systems NE (bioMérieux) microtest. The results were expressed as cfu per 100 mL.

Isolation and Identification of Legionella Strains

Analyses for the detection and quantification of Legionella species were performed in accordance with the ISO 11731 method.18 Hot water samples were previously concentrated 100-fold by filtration through a 0.2-µm-pore-size polycarbonate filter (Millipore). Serogrouping was performed by means of an agglutination test (Legionella latex test; Oxoid), which allows separate identification of L. pneumophila se-

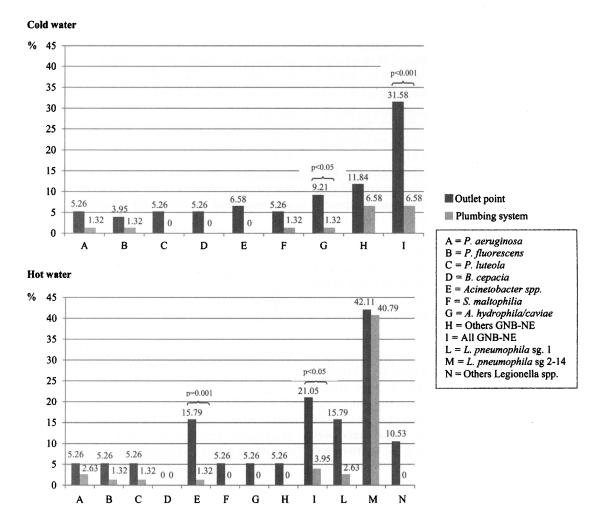


FIGURE 1. Percentage of samples positive for nonfastidious gram-negative bacteria (GNB-NE) in the cold and hot water system and Legionella in hot water system. sg, serogroup.

rogroup 1 and serogroups 2-14 as well as the detection of 7 species of Legionella (polyvalent) that have been implicated in human disease: L. longbeachae, L. bozemanii 1 and 2, L. dumoffii, L. gormanii, L. jordanis, L. micdadei, and L. anisa. The results were expressed as cfu per liter. According to this method, the lower limit of detection is 100 cfu/L.¹⁹

Statistical Analysis

Statistical analysis of the data was performed by means of Stata SE9TM software (Stata) using the nonparametric Wilcoxon matched-pairs signed-ranks test and Pearson χ^2 test to compare the microbiological quality of the water from the outlet points with that of the water from the plumbing system.

RESULTS

Microbiological assessment of the water entering the hospitals from the main supply did not reveal the presence of any GNB-NE in any of the samples taken. Moreover, the values

of HPCs at 36°C and 22°C and the concentrations of free chlorine proved to be within the target values proposed. 15,20

With regard to the cold water system, the outlet points displayed a higher percentage of positivity than did the rest of the plumbing system (Figure 1), with statistically significant differences being recorded only for GNB-NE as a whole (P < .001) and A. hydrophila/caviae (P < .05).

With regard to the concentration of the microbiological parameters analyzed, higher mean values were detected in the water samples taken at the outlet points than in the representative samples taken from the plumbing system (Table 1); the differences were always statistically significant. B. cepacia and Acinetobacter species reached maximum values of 1,875 cfu/100 mL and 360 cfu/100 mL, respectively, in water from outlet points, whereas they were never detected in the hospital plumbing system.

The mean values of the temperature and of the concentration of free chlorine in the cold water system proved to

TABLE 1. Heterotrophic Plate Counts (HPCs) at 22°C and 36°C and Nonfastidious Gram-Negative Bacteria (GNB-NE) Analyzed in Cold and Hot Water Systems and Legionella Species Analyzed in the Hot Water System

			Cold	Cold water				Hot water	ter	
	0	Outlet points	Plun	Plumbing system			Outlet points	ηΙ	Plumbing system	
Pathogen	Z	Mean ± SD	Z	Mean ± SD	Wilcoxon matched-pairs signed-ranks test	N	Mean ± SD	Z	Mean ± SD	Wilcoxon matched-pairs signed-ranks test
HPC 22°C (cfu/mL)	44,264	44,264 582.42 ± 849.12	4,212	55.42 ± 94.70	z = 7.485;	32,040	421.58 ± 482.20	7,316	96.26 ± 184.14	z = 7.577; $P < 0.01$
HPCs 36°C (cfu/mL)	37,128	37,128 488.53 ± 576.96	3,416 44.95	44.95 ± 72.80	z = 7.525; P < 0.01	29,688	390.63 ± 420.28	6,520	85.79 ± 91.00	z = 7.577; P < .001
GNB-NE (cfu/100 mL) Pseudomonas aeruginosa	357	4.70 ± 20.41	20	0.66 ± 5.74		843	11.09 ± 51.55	299	3.93 ± 24.28	z = 2.000; p = 0.005
Pseudomonas fluorescens	182	2.39 ± 10.37	21	0.28 ± 2.41		217	2.86 ± 13.29	14	0.18 ± 1.61	
Pseudomonas luteola	19	0.25 ± 1.21	0	0		24	0.32 ± 1.59	9	0.08 ± 0.69	2
Burkholderia cepacia	2,838	37.34 ± 232.77	0	0		0	0	0	0	1
Acinetobacter species	930	12.24 ± 57.54	0	0	121	3,278	43.13 ± 142.31	45	0.59 ± 5.16	z = 3.460;
Stenotrophomonas maltophilia	73	0.96 ± 5.01	11	0.14 ± 1.26	121	77	1.01 ± 5.37	0	0	
Aeromonas hydrophila/caviae	144	1.89 ± 5.87	7	0.03 ± 0.23	ا نتا	63	0.83 ± 4.26	0	0	z = 2.000;
Other GNB-NE	372	4.89 ± 20.01	9	0.08 ± 0.69		27	0.35 ± 1.65	0	0	ı % ∣
All GNB-NE	4,915	$4,915 64.67 \pm 255.14$	90	1.18 ± 6.32	z = 4.871; P < .001	4,529	59.59 ± 183.56	364	4.79 ± 28.56	P = .045 z = 3.911; P < .001
Legionella species (cfu/L) Legionella pneumophila	i	:	:	i	:	2,081,100	27,382.89 ± 42,245.33 1,479,100 19,461.84	1,479,100	19,461.84 ± 29,982.11	٠ . r., ،
Legionella pneumophila sg 1	÷	:	÷	i	:	48,400	$636.84 \pm 2,033.97$	200	6.58 ± 41.16	z = 3.460;
L. pneumophila sgs 2–14	i	÷	:	÷	i	2,032,700	26,746.05 ± 42,316.73	1,478,600	42,316.73 1,478,600 19,455.26 \pm 29,985.06	z = 5.592;
Other Legionella species	:		i	:	ï	2,700	35.53 ± 124.05	0	0	z = 2.827; P = .004

NOTE. cfu, colony-forming units; SD, standard deviation; sg, serogroup.

be $18.27^{\circ} \pm 1.96^{\circ}$ C (range, 15.2° – 21° C) and 0.04 ± 0.06 mg/L (range, 0.0–0.2 mg/L), respectively. On comparing the values of the HPCs at 22° C and 36° C and of *P. aeruginosa* with the target values (≤ 100 cfu/mL, ≤ 10 cfu/mL, and < 1 cfu/100 mL, respectively, as determined by the Ministère de la Santé et des Solidarités in 2007), a high percentage of nonconformity was recorded, especially with regard to HPCs at 36° C at the outlet points (94.74%; Figure 2).

With regard to the hot water system, the outlet points displayed a higher percentage of positivity than did the plumbing system, with statistically significant differences being recorded only for GNB-NE as a whole (P < .05) and for Acinetobacter species (P = .001). B. cepacia was not detected in any of the samples of hot water examined (Figure 1).

With regard to *L. pneumophila*, 47.36% and 42.11% of samples from outlet points and from the plumbing system, respectively, were positive. Specifically, serogroup 1 was detected in 15.79% of the samples from outlet points. In the plumbing system, the percentage was 2.63%; however, this difference did not prove significant. Almost half of the outlet points examined displayed contamination with serogroups 2–14, regardless of the presence (42.11%) or absence (40.79%) of an aerator (Figure 1).

Table 1 reports the mean values of the various microbiological parameters analyzed. Particularly high mean values of HPCs, at both 22°C and 36°C, were recorded in the hot water

system at outlet points, with maximum values of 1,600 cfu/mL and 1,840 cfu/mL, respectively.

With regard to *L. pneumophila*, the mean concentrations displayed statistically significant (P < .001) differences between the outlet points and the plumbing system. Specifically, *L. pneumophila* serogroups 2–14 displayed very high mean values, both in samples taken at outlet points and in those from the plumbing system, with maximum values of 180,000 cfu/L and 97,000 cfu/L, respectively. The mean concentrations were above the target value (10^3 cfu/L) and the alert value (10^4 cfu/L), and even within the action value ($>10^4$ cfu/L).

For all the microbiological parameters examined, the differences between the mean values at the outlet points and those of the plumbing system proved statistically significant. The mean values of temperature and of the concentration of free chlorine in the water system were $38.22^{\circ} \pm 8.08^{\circ}$ C (range, $26.1^{\circ}-53.6^{\circ}$ C) and 0.08 ± 0.25 mg/L (range, 0-1.1 mg/L), respectively.

Finally, in the hot water circuit, we calculated the percentages of samples containing concentrations of L. pneumophila within the target value ($<10^3$ cfu/L), the alert value ($>10^3$ and $<10^4$ cfu/L), and the action value ($>10^4$ cfu/L).^{15,21} It emerged that 42.11% and 38.16% of the samples from the outlet points and the plumbing system, respectively, fell within the level of action, whereas 3.95% and 2.63%, respectively, fell within the alert value.

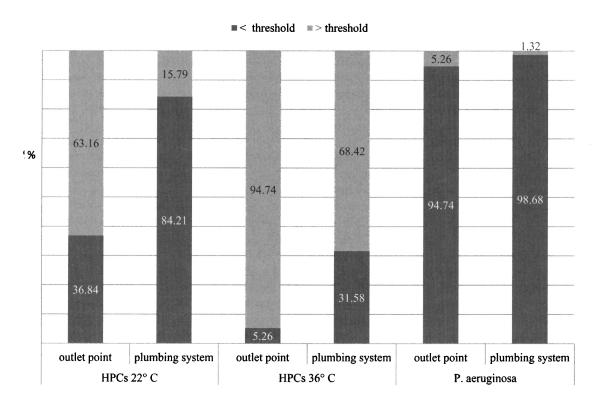


FIGURE 2. Percentage distribution of samples above and below target values for heterotrophic plate counts (HPCs) at 22°C (100 colony-forming units [cfu]/mL or less), HPCs at 36°C (10 cfu/mL or less), and *Pseudomonas aeruginosa* (less than 1 cfu/100 mL) in the cold water system.

When considered separately, the values of L. pneumophila serogroup 1 and L. pneumophila serogroups 2-14 in the plumbing system were found to be below 103 cfu/L in 100% and 59.21% of samples, respectively; with regard to samples from the outlet points, this percentage decreased to 90.79% and 57.89%, respectively.

DISCUSSION

Various studies have shown that aerators may be contaminated by gram-negative bacteria, including P. aeruginosa, S. maltophilia, B. cepacia, and A. calcoaceticus. In particular, aerators contaminated with P. aeruginosa or Pseudomonas species have been epidemiologically linked to colonized or infected patients.²² Wang et al²³ found that one-third of the ICU faucet aerators sampled were contaminated with other waterborne GNB-NE, in addition to P. aeruginosa; they also found a highly significant association between all of the GNB-NE studied and colonization and infection of ICU patients.

The aim of our study was to evaluate contamination by opportunistic waterborne gram-negative bacteria of the water in at-risk hospital facilities. We also compared the degree of contamination of water sampled at outlet points with that of the water in the plumbing system; this is an aspect which, to our knowledge, has not yet been investigated. The results of our research revealed, in both the cold and hot water systems, percentages of positivity and mean values of HPCs at 22°C and 36°C and GNB-NE loads that were significantly higher at water outlet points than in the plumbing system. In particular, the mean values of HPCs 22°C and HPCs 36°C in the samples taken from the outlet points of the cold water system proved to be 10-fold higher than the values recorded in water samples from the plumbing system (HPCs at 22°C, 582.42 cfu/mL vs 55.42 cfu/mL; HPCs at 36°C, 488.53 cfu/ mL vs 44.95 cfu/mL). With regard to the hot water system, a smaller difference in the values of the above-mentioned parameters was seen between the outlet points and the plumbing system.

No reference limits have been set on the concentrations of GNB-NE, with the exception of P. aeruginosa. 15 P. aeruginosa was detected in 5.26% of samples taken from the outlet points of both the cold and hot water systems, mean concentrations being 4.70 \pm 20.41 cfu/100 mL and 11.09 \pm 51.55 cfu/100 mL, respectively.

Apart from Legionella, the GNB-NE microorganism most frequently detected in the hot water system proved to be Acinetobacter species, a genus that has been implicated in various nosocomial outbreaks of waterborne disease in critical facilities.24,25 The contamination of water by GNB-NE may be explained by the capacity of these microorganisms to induce the development of biofilms.16 Because conventional aerators are made of several wire meshes, which collect the sediment present in the pipes and cause some water stagnation at the outlets, low colony counts that may be present in the water can increase over time.²⁴ The contamination of water by GNB-NE may also constitute an indirect risk for immunocompromised patients as a result of splashes from sinks, because it has been demonstrated that splashes can contaminate the surrounding environment.26

It is noteworthy that the mean concentration of free chlorine (0.04 \pm 0.06 mg/L in cold water and 0.08 \pm 0.25 mg/ L in hot water) proved to be approximately 5-fold and 2.5fold lower, respectively, than the value (0.2 mg/L) recommended by Italian legislation.²⁰ This finding indicates the consumption of disinfectant by the microbial load resident in the system. Moreover, very low concentrations of chlorine, like those recorded in our study, may constitute an additional healthcare risk in terms of the possible presence of multidrugresistant microbial strains of P. aeruginosa, which display greater resistance to chlorine than do susceptible strains.²⁷

L. pneumophila was searched for only in the hot water system, because the mean temperature of the cold water system (18.27°C) was below the value (20°C) that the CDC and Healthcare Infection Control Practices Advisory Committee guidelines recommend should be maintained in healthcare facilities to prevent the growth of Legionella and other bacteria.4 Our results revealed contamination by L. pneumophila both in the plumbing system and at outlet points, with percentages of positive samples varying according to the serogroup examined.

Serogroup 1 was mainly detected in the outlet points (15.79%), the mean concentrations (636.84 \pm 2,033.97 cfu/ L) being higher than in the water in the plumbing system (2.63% and 6.58 ± 41.16 cfu/L). Although this serogroup is the main causative agent of disease, it was isolated less frequently than the other serogroups (2-14) in our study, as in other studies.^{28,29} With regard to serogroups 2-14, the percentages of positivity proved to be very similar in the 2 types of water sample (42.11% vs 40.79%), although the mean values of contamination were significantly higher in samples from outlet points (26,746.05 cfu/L) than in those from the plumbing system (19,455.26 cfu/L). The results obtained seem to indicate that contamination by these serogroups can mainly be attributed to the water system itself and that the presence of aerators influences the concentration of the microorganism rather than the percentage of positive samples.

Italian guidelines do not regard water with a concentration of Legionella species less than 10³ cfu/L as being contaminated. However, they recommend clinical surveillance when levels of Legionella are between 103 and 104 cfu/L and the adoption of disinfection measures at levels greater than 10⁴ cfu/L.²¹ On the basis of these threshold values, which also correspond to those indicated by the French guidelines, 42.11% and 38.16% of our samples from outlet points and the plumbing system, respectively, reached the level at which disinfection measures are recommended.

Temperature is an important factor in controlling Legionella colonization. In the facilities investigated, the water temperatures were often below the minimum level recommended by the international³⁰ and national guidelines (at least 50°C at distal outlets and in recirculating water).³¹ In our study, the mean temperature of the hot water was 38.22°C, a value that probably favored the development not only of *Legionella* but also of the total microbial load, as previously demonstrated.

In the facilities examined, no cases of legionellosis were reported during the study period (1 year). However, the results of environmental sampling prompted healthcare management to undertake precautionary measures to reduce the legionellosis risk for patients. The World Health Organization^{32,33} guidelines recommend that all hospitals and other healthcare facilities adopt a water safety plan as part of their infection control program to reduce the number of healthcare-associated infections potentially acquired from water.

Regarding the contamination of water at outlet points, clear guidelines on the use of aerators in healthcare facilities have not been developed, beyond recommending monthly cleaning and disinfection in areas with high-risk patients to control *Legionella* species.⁸ However, some infection control experts have recommended regular cleaning of aerators or removal of aerators from high-risk areas;²⁴ others have pointed out that, although disinfecting aerators does reduce contamination, the efficacy of this measure is short lived.^{27,34}

In neither of the 2 hospitals monitored was a scheduled program of aerator maintenance implemented; aerators were replaced only when department staff reported anomalies in the flow of water. It was not therefore possible to correlate this variable with the results on microbial contamination, and this constitutes a limitation of our study.

Our results show that there is a possible risk of exposure of at-risk patients to waterborne opportunistic gram-negative microorganisms and that this risk could be attributable to the presence of inadequately maintained aerators. Thus, given the potential healthcare risk posed by aerators, in that they can constitute a reservoir and a source of infection, ^{22,24} there is clearly a need to place greater emphasis on the management of these devices. As has been pointed out by other authors, ¹⁴ safe water is vital to ensuring patient safety and reducing costs in settings where waterborne infections increase morbidity, mortality, treatment costs, and compensation claims and prolong hospital stays.

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