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Identification of Microorganisms Frequently Isolated in the Pharmaceutical Industry and Investigation of Their Sensitivity Against Various Disinfectants

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Identification of Microorganisms Frequently Isolated in the Pharmaceutical Industry and Investigation of Their Sensitivity Against Various Disinfectants

Summary : *In this study, the samples were taken by open dishe, swab and membrane filtration techniques from a company which manufactures pharmaceutical products in Turkey. Overall 255 bacterial strains were isolated from 112 samples; in manufacturing operators, dressing rooms, manufacturing areas, machines and equipments, water, microbiology laboratory and final products.*

Bacterial strains were identified according to their morphological and biochemical characteristics and 29 different bacterial species were determined. We found that 154 of the 255 isolated strains were coagulase negative Staphylococcus, 40 strains were Micrococcus, *44 strains were* Bacillus *and two strains were* Corynebacterium. *We also identified two* Pseudomonas aeruginosa, *two* Burkholderia cepacia, *one* Staphylococcus aureus, Xanthomonas maltophilia, Aeromonas sabria, Ochrobactrum anthropi *and* Meeksella zoohelcum *strains in the samples which were taken from the air of manufacturing areas,* Pseudomonas chlororaphis, X.maltophilia, A.sabria *and* Agrobacter radiobacter *from water samples and two* A.radiobacter *strains from final products.*

Using six different disinfectants which were routinely used at the pharmaceutical company, we assessed the inhibition coefficient, inferior lethal coefficient and superior lethal coefficient against 29 strains of different species. For this purpose we used benzalkonium chloride, a combination of 9 % of benzalkonium chloride and nonionic surface active agent, a mixture of 5 % of benzalkonium chloride, 1,35 % of formaldehyde, 0,75 % of glutaraldehyde and 3,8 % of glyoxal, a mixture of 0,1 % of glutaraldehyde, 32,5 % of 1-propanol and 18 % of ethanol, 10 % of sodium hypochloride and chloramine-T trihydrate.

Consequently, the findings of our study showed that sodium hypochloride and chloramine- T trihydrate showed no activity against most of the strains at a concentration of 1/100, all the other disinfectants showed bactericidal activity and the combination of 9 % benzalkonium chloride and nonionic surface

İlaç Sanayiinde Sıklıkla İzole Edilen Mikroorganizmaların Tanımı ve Bunların Dezenfektan Maddelere Karşı Duyarlılığının İncelenmesi

Özet : *Bu çalışmada ilaç üretimi sırasında meydana gelebilecek mikrobiyal kontaminasyonun kaynaklarını araştırmak üzere ülkemizdeki bir ilaç fabrikasının üretimde çalışan personelinden, soyunma odalarından, üretim alanlarından, makina ve ekipmanlarından, suyundan, mikrobiyoloji laboratuarından ve bitmiş ürünlerinden açık petri, swab ve membran filtrasyon yöntemleri ile örnekler alınmış, alınan 112 örnekten 255 adet bakteri suşu izole edilmiştir.*

İzole ettiğimiz suşların morfolojik ve biyokimyasal özelliklerine göre tanıları yapılmış ve bu bakterilerden 154'ünün koagülaz negatif stafilokok, *40'ının* mikrokok, *44'ünün* Bacillus, *ikisinin* Corynebacterium *cinsindeki türlere ait saprofit bakteriler olduğu saptanmıştır. Ayrıca üretim sahasının havasında bir adet* Staphylococcus aureus, *iki adet* Pseudomonas aeruginosa, *iki adet* Burkholderia cepacia, *birer adet* Xanthomonas maltophilia, Aeromonas sabria, Ochrobactrum anthropi *ve* Meeksella zoohelcum*; su örneklerinde birer adet* Pseudomonas chlororaphis, Agrobacter radiobacter, X.maltophilia *ve* A.sabria*; ürünlerde iki adet* A.radiobacter *suşu izole edilmiş ve bu bakterilerin toplam 29 farklı türe ait oldukları saptanmıştır.*

Tanısı yapılan 29 bakteri türünün aynı ilaç fabrikasında kullanılan dezenfektan maddelerden benzalkonyum klorür; %9 benzalkonyum klorür, noniyonik yüzey etken madde karışımı; %5 benzalkonyum klorür, %1.35 formaldehit, %0.75 glutaraldehit ve %3.8 glioksal karışımı; %0.1 glutaraldehit, %32.5 1-propanol ve %18 etanol karışımı; %10'luk sodyum hipoklorit ve kloramin-T trihidrata karşı duyarlılıkları araştırılarak inhibisyon, inferior ve superior letal katsayıları saptanmıştır.

Yapılan deneyler sonucunda serbest klor içeren bileşiklerden sodyum hipoklorit ve kloramin-T trihidratın izole ettiğimiz bakterilerin çoğunu 1/100 konsantrasyonda öldüremediği, diğer tüm dezenfektan maddelerin öldürücü et-

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active agent was the most active disinfectant against the strains which were isolated from a pharmaceutical company.

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kili olduğu ve %9 benzalkonyum klorür noniyonik yüzey etken madde karışımının en etkili dezenfektan madde olduğu saptanmıştır.

Anahtar kelimeler : *İlaç sanayii, mikrobiyal kontaminasyon, dezenfeksiyon, dezenfektan madde aktivitesi, bakterilerin izolasyonu ve tanımı.*

INTRODUCTION

As known, there are several microorganisms both saprophyte and pathogen or potential pathogen in nature, dust, soil, water and on the bodies of human and animals. These microorganisms grow and increase by using several materials in their living area when the convenient conditions are suitable. Thus, they have harmful effect on the structure of the material.

Many pharmaceutical products may be contaminated with widespread microorganisms in nature. This contamination may spring from raw materials, production machines and equipments, package materials, the air of production areas, water from several steps and manufacturing operators. Pharmaceutical products may also be contaminated when they are consumed or during their shelf life.

As a result of the microbial contamination, the structure stability and activity of the drug is damaged by physical and chemical changes. In the prospectus for this type of drugs, the possible consequence may not exist and also some new infections may appear with a high probability[1-3].

Such cases are prevented only by production of these drugs according to certain rules. These rules allow the drug to be qualified not only from microbiological but also all other aspects. These are the rules that were published by World Health Organization (WHO) in 1968 under the name of Good Manufacturing Practice (GMP) and since 1984 have been practiced in Turkey[4-6].

Preventing the microbial contamination of the drug is a matter of determining what the contaminants and the source of this contamination is. In our research, we tried to define the contamination sources and the contaminants with the samples from several areas in the pharmaceutical company. Then we investigated the sensitivity of these bacteria to the widely using disinfectants in the pharmaceutical company.

MATERIAL AND METHODS

Samples

From a company who manufactures pharmaceutical products in Turkey, 112 samples were taken of which 12 from manufacturing operators, 20 from dressing rooms, 26 from manufacturing areas, 10 from machines and equipments, three from water, three from microbiology laboratory and 38 from final products (vials that were filled for validation).

When these samples were taken the open dishe technique for samples from air, membrane filtration technique for liquid samples and swab technique for other samples were used.

Media

In our study Triptic Soy Agar (Difco) and Triptic Soy Broth (Difco) medium were used for isolating microorganisms and to determine the activities of disinfectants. In biochemical experiments to describe the isolated bacterial strains, some special mediums for Gram positive cocci and rods and API 20 E and API 20 NE (Biomérioux) kits for Gram negative rod bacteria were used.

Disinfectants

Using six different disinfectants which were routinely used at the pharmaceutical company we determined the inhibition coefficient, inferior lethal coefficient and superior lethal coefficient against isolated and identified bacterial strains[7-11]. For this reason, disinfectants (A, B, C, E and F) were diluted with sterile purified water between 1/10 - 1/100 concentrations and disinfectant D which is in aerosol form and used directly, diluted between 1 - 1/10 concentrations.

These disinfectants are:

A. A combination of 9 % of benzalkonium chloride and nonionic surface active agent (Micro-quat, Ecolab inc.)
B. A mixture of 5 % of benzalkonium chloride, 1,35

% of formaldehyde, 0,75 % of glutaraldehyde and 3,8 % of glyoxal (Diesin R-80, Henkel)
C. Benzalkonium chloride (Teknik)
D. A mixture of 0,1 % of glutaraldehyde, 32,5 % of 1-propanol and 18 % of ethanol (Aerodesin 2000, Flachen gegenstande)
E. 10 % of sodium hypochloride (Teknik)
F. Chloramine- T trihydrate (Merck)

RESULTS AND DISCUSSION

Overall 255 pure cultures were isolated from 112 samples. Bacterial strains were identified according to their morphological and biochemical characteristics and 29 different bacterial species were determinated. The number of these bacteria and their distrubutions according to the isolation areas are given in Table 1 and isolated bacterial species are given in Table 2-8.

Table 1. Distribution of Bacteria According to Their Isolation Areas

	Gram positive cocci		Gram positive rods		Gram negative rods	
Isolation areas of bacteria	number	%	number	%	number	%
Manufacturing operators	23	12	2	4	-	-
Dressing rooms	40	20	5	11	-	-
Manufacturing areas	67	34	18	39	8	57
Machine and equipments	15	8	14	31	-	-
Water	-	-	2	4	4	29
Microbiology laboratory	15	8	1	2	-	-
Final products	35	18	4	9	2	14
Total	195	100	46	100	14	100

Table 2. Bacteria Isolated From Manufacturing Operators

Manufacturing operators	Number of samples	number	Bacteria species
Hands	5	3	Staphylococcus cohnii subs. ureolyticum
		2	Staphylococcus intermedius
		2	Staphylococcus pisciferm entans
		1	Staphylococcus simulans
		1	Micrococcus roseus
		1	Bacillus pumilus
		1	Corynebacterium
Arms	4	4	Staphylococcus pisciferm entans
		1	Staphylococcus intermedius
		1	Staphylococcus capitis subs. ureolyticus
Neck	2	1	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus capitis subs. ureolyticus
		1	Staphylococcus intermedius
		1	Staphylococcus pisciferm entans
		1	Micrococcus roseus
		1	Micrococcus varians
Dress	1	1	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus equorum
Total	12	25	

Table 3. Bacteria Isolated From Water

Water	number of samples	number	bacteria species
Deionized water	2	1	Agrobacter radiobacter
		1	Aeromonas sabria
		1	Pseudomonas chlororaphis
Flask water	1	1	Bacillus subtilis
		1	Bacillus pumilus
		1	Xanthomonas maltophilia
Total	3	6	

Table 4. Bacteria Isolated From Dressing Rooms of Manufacturing Operators

Dressing rooms	number of samples	number	bacteria species
Air	5	4	Staphylococcus capitis subs. ureolyticus
		3	Staphylococcus cohnii subs. ureolyticum
		2	Staphylococcus intermedius
		1	Staphylococcus saprophyticus
		2	Micrococcus roseus
		2	Micrococcus varians
		1	Micrococcus nishinomiyaensis
		1	Bacillus pumilus
		1	Bacillus polymyxa
		1	Bacillus licheniformis
Floor	2	2	Staphylococcus intermedius
		1	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus capitis subs. ureolyticus
		1	Staphylococcus saprophyticus
		1	Staphylococcus pisciferm entans
		1	Staphylococcus equorum
		1	Micrococcus varians
Wall	2	1	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus simulans
Working surfaces	2	1	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus saprophyticus
		1	Micrococcus varians
		1	Corynebacterium
Hangers	2	2	Staphylococcus saprophyticus
		1	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus intermedius
		1	Staphylococcus pisciferm entans
		1	Micrococcus roseus
		1	Bacillus pumilus
Door handle	2	1	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus saprophyticus
		1	Staphylococcus pisciferm entans
		1	Staphylococcus equorum
Glove hanger	3	-	-
Mirror	1	1	Staphylococcus capitis subs. ureolyticus
Window	1	1	Micrococcus roseus
Total	20	45	

Table 5. Bacteria Isolated From Manufacturing Areas

Manufacturing areas	number of samples	number	bacteria species
Air	10	11	Staphylococcus cohnii subs. ureolyticum
		9	Staphylococcus intermedius
		8	Staphylococcus capitis subs. ureolyticus
		1	Staphylococcus piscifermentans
		1	Staphylococcus caseolyticus
		1	Staphylococcus aureus
		6	Micrococcus varians
		4	Micrococcus roseus
		1	Micrococcus nishinomiyaensis
		4	Bacillus pumilus
		1	Bacillus polymyxa
		1	Bacillus subtilis
		2	Pseudomonas aeruginosa
		2	Burkholderia cepacia
		1	Aeromonas sabria
		1	Ochrobactrum anthropi
		1	Meeksella zoohelcum
		1	Xanthomonas maltophilia
Floor	9	2	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus saprophyticus
		1	Staphylococcus gallinarum
		1	Micrococcus varians
		2	Bacillus subtilis
		2	Bacillus pumilus
		1	Bacillus licheniformis
Working surfaces	3	3	Staphylococcus cohnii subs. ureolyticum
		3	Staphylococcus capitis subs. ureolyticus
		3	Staphylococcus intermedius
		1	Staphylococcus saprophyticus
		2	Micrococcus roseus
		2	Micrococcus varians
		4	Bacillus polymyxa
		2	Bacillus pumilus
		1	Bacillus brevis
Steel stool	3	2	Staphylococcus cohnii subs. ureolyticum
		2	Staphylococcus intermedius
Alcohol vessel	1	1	Staphylococcus cohnii subs. ureolyticum
		1	Micrococcus nishinomiyaensis
Total	26	93	

Table 6. Bacteria Isolated From Manufacturing Areas

Machines and equipments	number of samples	number	bacteria species
Surface of machines	5	2	Staphylococcus capitis subs. ureolyticus
		1	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus saprophyticus
		1	Staphylococcus intermedius
		1	Micrococcus varians
		9	Bacillus polymyxa
		2	Bacillus brevis
		2	Bacillus firmus
		1	Bacillus licheniformis
Top of machines	2	1	Staphylococcus intermedius
		2	Micrococcus nishinomiyaensis
Trunks	2	2	Staphylococcus intermedius
		1	Staphylococcus cohnii subs. ureolyticum
		1	Micrococcus varians
		1	Micrococcus nishinomiyaensis
		1	Staphylococcus intermedius
Total	10	29	

Table 7. Bacteria Isolated From Microbiology Laboratory

Microbiology laboratory	number of samples	number	bacteria species
Surface of working areas	2	4	Staphylococcus cohnii subs. ureolyticum
		1	Staphylococcus intermedius
		2	Micrococcus varians
		2	Micrococcus nishinomiyaensis
		1	Bacillus polymyxa
	1	3	Staphylococcus cohnii subs. ureolyticum
Sides of laminar air flow		1	Staphylococcus intermedius
		1	Staphylococcus caseolyticus
		1	Micrococcus nishinomiyaensis
Total	3	16	

Table 8. Bacteria Isolated From Final Products

Final products	number of samples	number	bacteria species
Final products	38	9	Staphylococcus cohnii subs. ureolyticum
		6	Staphylococcus equorum
		7	Staphylococcus piscifermentans
		6	Staphylococcus simulans
		4	Staphylococcus saprophyticus
		3	Staphylococcus intermedius
		4	Bacillus pumilus
		2	Agrobacter radiobacter
Total	38	41	

Against the isolated bacteria, lethal coefficients of disinfectants are given in Table 9; superior lethal coefficients are given in Table 10 and inhibition coefficients are given in Table 11.

FDA (Food and Drug Administration) has described microorganisms under three groups; saprophyte, pathogen and potential pathogen. Some infections in humans are caused by pathogen microorganisms or their toxins. Saprophyte microorganisms do not cause any infection. Potential pathogen microorganisms may cause some infections in human whose immune systems are damaged. This type of microorganisms can be found in many raw and auxillary materials which are used either in drug manufacturing or in manufacturing areas[2,3,5].

Most of the microorganisms which can be found in manufacturing areas are Gram positive rods, Gram positive cocci, molds and yeasts. Gram negative rods are also found in different areas of the phar-

Table 9. Inferior Lethal Coefficients of Disinfectants Against Gram Positive Cocci, Gram Negative Rods and Gram Positive Rods

	A		B		C		D		E		F	
BACTERIA	CON.	MIN.	CON.	MIN.	CON.	MIN.	CON.	MIN.	CON.	MIN.	CON.	MIN.
S. cohnii subs ureolyticum	1/1000	2,5'	1/600	7,5'	1/400	2,5'	1/5	2,5'	>1/100	10'	>1/100	10'
S. intermedius	1/1000	2,5'	1/1000	2,5'	1/1000	2,5'	1/9	2,5'	>1/100	10'	1/200	7,5'
S. capitis subs ureolyticus	1/900	2,5'	1/20	2,5'	1/500	2,5'	1/3	5'	1/100	7,5'	>1/100	10'
S. pisciferm entans	1/1000	2,5'	1/1000	2,5'	1/500	2,5'	1/3	2,5'	>1/100	10'	1/200	7,5'
S. saprophyticus	1/1000	2,5'	1/1000	2,5'	1/1000	2,5'	1/10	2,5'	1/200	10'	1/200	10'
S. equorum	1/1000	2,5'	1/300	2,5'	1/400	2,5'	1/5	10'	>1/100	10'	>1/100	10'
S. simulans	1/900	2,5'	1/400	2,5'	1/500	2,5'	1/5	7,5'	>1/100	10'	1/200	2,5'
S. caseolyticus	1/1000	2,5'	1/1000	2,5'	1/1000	2,5'	1/8	2,5'	1/100	5'	1/100	2,5'
S. aureus	1/1000	10'	1/800	10'	1/600	5'	1/4	2,5'	>1/100	10'	>1/100	10'
S. gallinarum	1/1000	10'	1/200	2,5'	1/200	2,5'	1/4	2,5'	>1/100	10'	>1/100	10'
M. varians	1/1000	2,5'	1/1000	2,5'	1/1000	2,5'	1/5	2,5'	>1/100	10'	1/500	5'
M. roseus	1/1000	2,5'	1/1000	2,5'	1/1000	2,5'	1/5	5'	>1/100	10'	>1/100	10'
M.nishinomiyaenois	1/1000	2,5'	1/1000	2,5'	1/1000	2,5'	1/9	2,5'	1/300	2,5'	1/300	2,5'
Agrobacter radiobacter	1/700	10'	1/300	2,5'	1/400	10'	1/4	10'	>1/100	10'	>1/100	10'
Aeromonas sabria	1/700	2,5'	1/400	2,5'	1/200	2,5'	1/4	10'	>1/100	10'	>1/100	10'
Pseudomonas aeruginosa	1/600	10'	1/400	5'	1/200	2,5'	1/5	10'	>1/100	10'	>1/100	10'
Pseudomonas chlororaphis	1/1000	2,5'	1/1000	2,5'	1/1000	2,5'	1/9	2,5'	>1/100	10'	>1/100	10'
Burkholderia cepacia	1/1000	2,5'	1/1000	10'	1/500	5'	1/3	7,5'	>1/100	10'	>1/100	10'
Xanthomonas maltophilia	1/1000	2,5'	1/1000	2,5'	1/700	10'	1/4	7,5'	>1/100	10'	>1/100	10'
Meeksella zoohelcum	1/1000	5'	1/1000	2,5'	1/700	2,5'	1/7	7,5'	>1/100	10'	>1/100	10'
Ochrobactrum anthropi	1/1000	2,5'	1/1000	2,5'	1/700	2,5'	1/5	7,5'	>1/100	10'	>1/100	10'
Corynebacterium	1/900	2,5'	1/400	2,5'	1/1000	2,5'	1/3	2,5'	1/500	2,5'	>1/100	10'

CON : Concentration MIN : Minute

A, B, C, D, E, F : Disinfectants Mentioned in Material and Methods

Table 10. Superior Lethal Coefficients of Disinfectants Against Gram Positive Rods

	A		B		C		D		E		F	
BACTERIA	CON.	MIN.	CON.	MIN.	CON.	MIN.	CON.	MIN.	CON.	MIN.	CON.	MIN.
B. polymyxa	1/500	2,5'	1/200	10'	1/500	2,5'	1/2	2,5'	>1/100	10'	>1/100	10'
B. pumilus	1/500	7,5'	1/100	2,5'	1/400	2,5'	1/3	2,5'	>1/100	10'	>1/100	10'
B. subtilis	1/500	2,5'	1/200	2,5'	1/500	2,5'	1/4	7,5'	>1/100	10'	>1/100	10'
B. brevis	1/700	7,5'	1/1000	7,5'	1/400	2,5'	1/3	2,5'	>1/100	10'	>1/100	10'
B. licheniformis	1/500	2,5'	1/100	2,5'	1/300	2,5'	1/3	10'	>1/100	10'	>1/100	10'
B. firmus	1/600	7,5'	1/1000	7,5'	1/200	2,5'	1/3	10'	>1/100	10'	>1/100	10'
B. coagulans	1/500	2,5'	1/200	2,5'	1/500	2,5'	1/2	2,5'	>1/100	10'	>1/100	10'

CON : Concentration MIN : Minute

A, B, C, D, E, F : Disinfectants Mentioned in Material and Methods

Table 11. Inhibition Coefficients of Disinfectants Against Gram Positive Cocci, Gram Negative Rods and Gram Positive Rods

	A		B		C		D		E		F	
BACTERIA	I.C.	L.C.	I.C.	L.C.	I.C.	L.C.	I.C.	L.C.	I.C.	L.C.	I.C.	L.C.
S. cohnii subs ureolyticum	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/7	1/5	>1/100	>1/100	>1/100	>1/100
S. intermedius	</1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/10	1/10	>1/100	>1/100	1/300	1/200
S. capitis subs ureolyticus	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/5	1/3	1/200	1/100	>1/100	>1/100
S. piscifermentans	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/4	1/3	>1/100	>1/100	1/200	1/200
S. saprophyticus	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/10	1/10	1/200	1/100	1/200	1/100
S. equorum	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/6	1/4	>1/100	>1/100	>1/100	>1/100
S. simulans	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/7	1/4	>1/100	>1/100	1/200	1/200
S. caseolyticus	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/10	1/8	1/200	1/100	1/100	1/100
S. aureus	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/5	1/3	>1/100	>1/100	>1/100	>1/100
S. gallinarum	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/6	1/4	>1/100	>1/100	>1/100	>1/100
M. varians	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/7	1/5	>1/100	>1/100	1/500	1/500
M. roseus	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/7	1/5	>1/100	>1/100	>1/100	>1/100
M. nishinomiyaenois	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/10	1/10	1/400	1/300	1/300	1/300
Agrobacter radiobacter	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/7	1/4	>1/100	>1/100	>1/100	>1/100
Aeromonas sabria	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/6	1/4	>1/100	>1/100	>1/100	>1/100
Pseudomonas aeruginosa	1/600	1/500	1/900	1/800	1/700	1/500	1/7	1/5	>1/100	>1/100	>1/100	>1/100
Pseudomonas chlororaphis	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/10	1/10	>1/100	>1/100	>1/100	>1/100
Burkholderia cepacia	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/6	1/4	>1/100	>1/100	>1/100	>1/100
Xanthomonas maltophilia	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/6	1/5	>1/100	>1/100	>1/100	>1/100
Meeksella zoohelcum	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/10	1/7	>1/100	>1/100	>1/100	>1/100
Ochrobactrum anthropi	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/7	1/5	>1/100	>1/100	>1/100	>1/100
B. polymyxa	<1/1000	>1/100	<1/1000	>1/100	<1/1000	1/100	1/10	1/2	>1/100	>1/100	>1/100	>1/100
B. pumilus	<1/1000	>1/100	<1/1000	>1/100	<1/1000	1/100	1/10	1/3	>1/100	>1/100	>1/100	>1/100
B. subtilis	<1/1000	>1/100	<1/1000	>1/100	<1/1000	1/100	1/10	1/3	>1/100	>1/100	>1/100	>1/100
B. brevis	<1/1000	1/200	<1/1000	1/400	<1/1000	1/100	1/10	1/3	>1/100	>1/100	>1/100	>1/100
B. licheniformis	<1/1000	>1/100	<1/1000	>1/100	<1/1000	1/100	1/10	1/2	>1/100	>1/100	>1/100	>1/100
B. firmus	<1/1000	1/300	<1/1000	1/300	<1/1000	1/100	1/10	1/2	>1/100	>1/100	>1/100	>1/100
B. coagulans	<1/1000	>1/100	<1/1000	>1/100	<1/1000	>1/100	1/10	1/2	>1/100	>1/100	>1/100	>1/100
Corynebacterium	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	<1/1000	1/10	1/3	1/600	1/500	>1/100	>1/100

I.C : Inhibitor Concentration L.C : Lethal Concentration

A, B, C, D, E, F : Disinfectants Mentioned in Material and Methods

in natural raw materials. In this study we found that 155 (76 %) of 255 bacterial strains were Gram positive cocci, 46 (18 %) were Gram positive rods and 14 (6 %) were Gram negative rods, isolated from different samples taken from different areas and manufacturing operators in the company. It is determined that most of the isolated Gram positive cocci were coagulase negative *Staphylococcus* and *Micrococcus* species. In addition, from an air sample which was taken in one of the manufacturing area we isolated one *Staphylococcus aureus* strain. It is also determined that most of the Gram positive cocci were *Bacillus* species and two were *Corynebacterium* species.

Both *coagulase negative Staphylococcus, Micrococcus* and *Bacillus* species are saprophyte bacteria that can be widely found in nature, in dust, soil and the air and generally they do not cause any infection. On the other hand, it is known that *S.aureus* causes various type of infections in humans[3,12-14]. The isolated Gram negative rods are also potential pathogen bacteria which can cause infections. Among those bacteria, especially, *Pseudomonas aeruginosa, Burkholderia cepacia* and *Xanthomonas malthophilia* cause many types of serious infections[15-19].

roorganisms from different drug manufacturing areas as we did in this study. Disinfection procedures are carried out in order to prevent the contamination of the product with saprophyte, pathogen and potential pathogen microorganisms which can be exist in the environment during production of the pharmaceutical product, and to decrease their number in the environment. For this purpose various chemical disinfectants are used[20-23]. The effect of disinfectants on the growth of microorganisms may be static or cidal, depending on species, number and physical state of the microorganisms; type, concentration and contact time of the disinfectants and characteristics of the surroundings.

Gram negative rods are usually more resistant than Gram positive bacteria to disinfectants. Potential pathogens such as *P.aeruginosa* and *B.cepacia* are the most resistant Gram negative rods to disinfectants, which may not have their usually lethal effect against these bacteria. In our study, we determined that the disinfectants which we tested had less activity on Gram negative rods bacteria than other saprophyte bacteria.

Although there are a number of studies on resistance of bacteria to antibiotics, researches about resistance of bacteria to disinfectants are very few[24]. In most disinfectant related studies, the activities on microorganisms are researched. With small differences between the methods, the experimental conditions, and the resistance of bacterial strains can significantly affect the activity of disinfectants. For this reason, it is difficult to compare the results of different studies[25].

In our study, it is determined that the results we obtained about activities of disinfectants have similarities with international and national research results. However, we think that the reason behind the low activities we obtained from the experiments especially for sodium hypochloride and chloramine- T trihydrate, which are compounds of chloride, are related to pH and stability. As a result of our study, we established that the most commonly used disinfectant materials in the drug company we study with, the mixture of 9 % benzalkonium chloride and nonionic surface active agent, is the most effective disinfectant material against all bacteria isolated from different parts of the pharmaceutical company.

CONCLUSION

When we investigated the related studies we could not find any study in which the microbial flora of a pharmaceutical company has been established. We determined that the microorganisms which are isolated from different part of manufacturing areas show differences in sensitivity against antiseptics and disinfectants. For this reason, each pharmaceutical company should select the most suitable disinfectant according to the established microbial flora and determine criteria such as dilution factors and contact time of the disinfectants. It is important to keep in mind that the random use of disinfectants can cause development of resistant microorganisms. In this study, we determined the microbial flora of a pharmaceutical company in Istanbul and made an evaluation to determine the most suitable policy for using disinfectant materials.

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