BACTERIOLOGY OF HEMODIALYSIS FLUIDS: ARE CURRENT PRACTICES MEANINGFUL?

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INTRODUCTION:

CURRENT PRACTICES FOR MONITORING BACTERIAL CONTAMINATION OF FLUIDS EMPLOYED IN THE PREPARATION AND EXECUTION OF HEMODIALYSIS THERAPY ARE DICTATED BY THE AAMI STANDARDS (1). THE MINIMUM QUALITY CONTROL OF BACTERIOLOGICAL CONTAMINATION IS MANDATED TO CONSIST OF A MONTHLY CULTURE OF WATER AND DIALYSATE AND/OR AN ENDOTOXIN TEST OF REPROCESSOR WATER. THE MAXIMUM CONTAMINATION LEVELS OF BACTERIA ARE SET AT 200 CFU/ML FOR REPROCESSOR WATER AND 2000 CFU/ML FOR DIALYSATE (1). ENDOTOXIN MAXIMUM CONCENTRATIONS ARE DEFINED AT 1 NG/ML, BUT ARE SPECIFIED FOR REPROCESSOR WATER ONLY. THESE GUIDELINES APPEAR TO HAVE SERVED QUITE WELL SINCE THE INCEPTION OF WIDE SPREAD HEMODIALYSIS THERAPY, FOR THERE HAVE BEEN INFREQUENT REPORTS OF DIALYSIS RELATED ENDOTOXEMIA OR SEPSIS. THE INTRODUCTION OF HIGH FLUX MEMBRANES WITH PERMEABILITIES TO 65000 DALTONS, HOWEVER, HAS INTRODUCED GREATER POTENTIAL FOR ENDOTOXEMIC REACTIONS AS A RESULT OF HIGHLY CONTAMINATED DIALYSATE. INDEED, THE FREQUENCY OF ENDOTOXIC REACTION IS REPORTED TO BE INCREASING (2). WE, AND OTHERS, (3,4) HAVE SHOWN THAT THE TRADITIONAL CULTURES ARE LIKELY TO UNDERESTIMATE THE BACTERIAL BURDEN OF PURIFIED WATER BY AS MUCH AS THREE ORDERS OF MAGNITUDE. ADDITIONALLY, BLAND ET. AL. (5) SUGGESTED THAT TRADITIONAL CULTURES WERE INADEQUATE FOR ASSESSING THE CONTAMINATION OF BICARBONATE CONCENTRATES (BCC) AND DIALYSATE SOLUTIONS. THEY SUGGESTED THAT THE PRIMARY CONTAMINANTS OF DIALYSATE ARE HALODURIC SPECIES AND REQUIRE THE ADDITION OF SODIUM CHLORIDE AND/OR BICARBONATE TO CULTURES TO RECOVER THE MAXIMUM NUMBER OF CONTAMINATING ORGANISMS (5). SIMILARLY, EBBEN ET. AL. (6) OBSERVED

HIGH LEVELS OF CONTAMINATION OF BOTH COMMERCIAL BCC AND LABORATORY PREPARED BCC. THEY TERM THE CONTAMINANTS HALOTOLERANT. MAN ET. AL. (8) SUGGESTED THAT BICARBONATE POWDERS ARE HIGHLY CONTAMINATED WITH BACTERIA, A POTENTIAL SOURCE FOR SEEDING THE CONCENTRATE. BLAND ET. AL. (5) REPORTED HEAVY CONTAMINATION OF COMMERCIALLY PREPARED BCC, ESPECIALLY IF INADEQUATE SANITIZATION OF STORAGE TANKS IS NOT FOLLOWED.

IN A PREVIOUS REPORT, WE OBSERVED THAT THERE WAS NO CORRELATION IN THE LEVEL OF VIABLE BACTERIAL CONTAMINATION AND THE AMOUNT OF ENDOTOXIN PRESENT IN WATER OR DIALYSATE (7). THE SHIFT TO BICARBONATE BUFFERING OF DIALYSATE HAS COMPUNDED THE POTENTIAL FOR HIGH BACTERIAL AND/OR ENDOTOXIN CONTAMINATION OF PREPARED DIALYSIS FLUID, AS REPORTED BY SEVERAL AUTHORS (5,6, 8). OBSERVATIONS OF ENDOTOXIC REACTIONS AND RISES IN CERTAIN MARKERS FOR WHITE CELL ACTIVATION ( ) SUGGEST THAT THE NEED FOR BETTER UNDERSTANDING OF THE BACTERIOLOGY OF DIALYSIS FLUIDS AND BETTER MONITORING OF CONTAMINATION OF THESE FLUIDS MAY BE MANDATED BY THE GROWING USE OF HIGH FLUX DIALYSIS THERAPY.

WE HAVE COMPARED THE RECOVERY OF BACTERIAL CONTAMINANTS AND IDENTIFIED THE SPECIES PRESENT IN THE THREE DIALYSIS ASSOCIATED FLUIDS THAT POSE SIGNIFICANT BACTERIOLOGICAL RISK TO THE HEMODIALYSIS PATIENT, NAMELY BCC, PURIFIED WATER AND PREPARED DIALYSATE. IN THE FIRST PART OF THIS STUDY WE TESTED THE HYPOTHESIS THAT DIALYSIS FLUIDS REQUIRE SALT SUPPLEMENTED MEDIA FOR MAXIMUM RECOVERY OF CONTAMINATING BACTERIA. WE EMPLOYED FIVE DIFFERENT MEDIA FOR THE RECOVERY OF THE CONTAMINANTS OF THESE FLUIDS. OUR RESULTS SUGGEST THAT DIALYSIS CENTER PREPARED BICARBONATE CONCENTRATES ARE SOMEWHAT BETTER WHEN CULTURED ON SALT SUPPLEMENTED MEDIA, WITH INFREQUENT CONTAMINANTS THAT GROW TO ONE OR TWO LOGS HIGHER COUNTS. THE DATA

ALSO SUGGESTS, HOWEVER, THAT BBC CAN BE KEPT RELATIVELY FREE OF BACTERIAL CONTAMINATION IF GOOD SANITIZATION PROCEDURES ARE FOLLOWED.

IN A PREVIOUS PAPER (3) WE REPORTED BETTER RECOVERY OF CONTAMINATING BACTERIA IN WATER AND DIALYSATE ON R2A THAN ON STANDARD METHODS OR TRYPTIC SOY MEDIUM CONTAINING GLUCOSE. THESE OBSERVATIONS HAVE BEEN EXTENDED AND CONFIRMED IN A MULTI-CENTER SAMPLING.

WE SELECTED ALL OF THE DISTINCT COLONIAL FORMS OF A FEW PAIRED SAMPLES OF WATER, BCC AND DIALYSATE IN THIS FIRST SERIES OF SAMPLES FOR ISOLATION AND IDENTIFICATION OF THE CONTAMINATING BACTERIA. BACTERIA RECOVERED AND IDENTIFIED FROM THESE PAIRED SAMPLES OF BCC, DIALYSATE AND WATER ARE VARIABLE ENOUGH TO SUGGEST THAT CONTAMINATION OF DIALYSATE FROM SOURCES OTHER THAN BCC AND WATER ARE NOT INFREQUENT AND MAY CALL FOR MORE RIGOUROUS ASEPTIC PROCEDURES IN THE DIALYSIS UNIT.

IN THE SECOND SERIS OF EXPERIMENTS WE COMPARED THE RECOVERY OF CONTAMINATING BACTERIA IN WATER AND DIALYSATE ON THE FOUR MEDIA RECOMMENDED BY THE AAMI STANDARD (1) FOR MONITORING OF BACTERIAL CONTAMINANTS WITH TSA CONTAINING GLUCOSE AND R2A MEDIUM, A MEDIUM FOR HIGH RECOVERY OF WATER CONTAMINATING ORGANISMS. WE FOUND THAT _____MEDIUM PRODUCED A STATISTICALLY HIGHER RECOVERY OF BACTERIA FOR PURIFIED WATER AND _____MEDIUM PRODUCED A STATISTICALLY HIGHER RECOVERY FROM PAIRED DIALYSATE SAMPLES.

R2A AGAR, STANDARD METHODS AGAR (SMA) AND TRYPTIC SOY AGAR (TSA) WERE PURCHASED FROM DIFCO LABORATORIES AND WERE PREPARED IN DEIONIZED GLASS DISTILLED WATER BY THE DIRECTIONS OF THE SUPPLIER. DEHYDRATED TRYPTIC SOY BROTH POWDER CONTAINING GLUCOSE WAS PURCHASED FROM SIGMA CHEMICAL CO., ST. LOUIS, MO AND AGAR MEDIUM WAS PREPARED SIMILARLY BY THE ADDITION OF L% AGAR TO PRODUCE A GLUCOSE SUPPLEMENTED

TRYPTIC SOY MEDIUM (TSBA). TWO PERCENT BY WEIGHT OF REAGENT GRADE SODIUM CHLORIDE (FISHER CHEMICAL CO., CINCINNATI, OH) WAS ADDED TO TSBA (TSBA-HS) AND TO STANDARD METHODS AGAR (SM-HS) TO PRODUCE HIGH SALT MEDIA. MILLIPORE SPC TOTAL RECOVERY CASSETTES WERE PURCHASED FROM MILLIPORE, INC., BEDFORD, MA. API NFT IDENTIFICATION SYSTEM KITS AND REAGENTS WERE PURCHASED FROM SHERWOOD MEDICAL, INC., ST. LOUIS, MO. STERILE PETRI PLATES AND STYRENE TEST TUBES WERE PURCHASED FROM FISHER SCIENTIFIC, INC. CINCINNATI, OHIO.

DIALYSATE, WATER AND BCC SAMPLES WERE COLLECTED FROM SEVERAL REGIONAL DIALYSIS CENTERS IN CENTRAL KENTUCKY, EAST KENTUCKY AND SOUTH CENTRAL INDIANA. DIALYSATE SAMPLES WERE COLLECTED FROM THE DIALYZER INLET OF DIALYSIS PROPORTIONERS IMMEDIATELY BEFORE PATIENT THERAPY WAS BEGUN OR WITH A STERILE DISPOSABLE SYRINGE FROM A LEUR SAMPLE PORT, IF PATIENTS WERE ON DIALYSIS. TWO SYSTEMS SAMPLED HAD CENTRAL DIALYSIS PREPARATION SYSTEMS. IN THESE CENTERS DIALYSATE WAS TAKEN WITH A STERILE DISPOSABLE PIPET FROM THE CENTRAL DIALYSATE RESERVOIR. WATER WAS COLLECTED BY CLEAN CATCH TECHNIQUE FROM SAMPLE PORTS ON THE CENTRAL WATER SYSTEM AFTER DISINFECTION AND FLUSHING WITH 2 OR MORE LITERS OF WATER. BICARBONATE CONCENTRATES WERE COLLECTED FROM CENTRAL PREPARATION/STORAGE TANKS OR FROM AN INDIVIDUAL JUG CONNECTED TO THE DIALYSIS PROPORTIONER, FROM WHICH DIALYSATE WAS COLLECTED. THE SAMPLES WERE RETURNED TO THE LABORATORY ON ICE AND WERE PROCESSED WITHIN 4 HOURS AFTER COLLECTION.

ANALYSIS OF SALT SUPPLEMENTED MEDIA:

CULTURES WERE PREPARED AT TWO OR MORE LOGS OF CONCENTRATION IN DUPLICATE BY THE POUR PLATE METHOD. FOR BCC CULTURES ENOUGH STERILE BCC WAS ADDED TO EACH PLATE TO PRODUCE A FINAL BICARBONATE

OF 1 ML IN 10 ML OF MEDIUM (RENAL SYSTEMS FORMULATION, 650 GM SODIUM BICARBONATE IN 8 L WATER). ALL CULTURES WERE INCUBATED FOR 96 HOURS IN AN AMBIENT TEMPERATURE (23°C) HUMIDIFIED CHAMBER (3). PLATES WERE COUNTED MANUALLY UNDER 5-10 POWER MAGNIFICATION. SINCE PLATES WERE PAIRED PLATINGS, PLATES THAT HAD LESS THAN 20 OR MORE THAN 200 CFU WERE NOT REJECTED UNLESS A MORE DILUTE PLATE COULD BE COUNTED RELIABLY.

IDENTIFICATION OF CONTAMINANTS IN PAIRED SAMPLES:

ALL OF THE DISTINCT COLONIES OF CERTAIN PAIRED SAMPLINGS WERE ISOLATED FRO IDENTIFICATION OF THE CONTAMINATING SPECIES. IDENTIFICATIONS WERE ACCOMPLISHED WITH API NFT STRIPS. IDENTIFICATIONS WERE MADE BY COMPARISON OF E.O. KING'S TABLES FOR IDENTIFICATION OF UNUSUAL GRAM NEGATIVE PATHOGENS (9). A CORRELATION OF THE CONTAMINATING SPECIES IN THE THREE FLUIDS WAS SOUGHT AND LINEAR REGRESSION ANALYSIS OF THE CONTAMINATION LEVELS IN BCC AND WATER VERSUS DIALYSATE WAS PERFORMED. LINEAR REGRESSION ANALYSIS OF WATER-DIALYSATE AND BCC-DIALYSATE WAS PERFORMED ON THE SET OF PAIRED SAMPLES TO DETERMINE IF ANY CORRELATION EXISTS IN THE COLONY COUNTS ON ANY OF THE MEDIA OR ON THE HIGHEST OBSERVED COUNTS OF ALL THE MEDIA.

COMPARISON OF FOUR MEDIA RECOMMENDED BY AAMI WITH TSA (GLUCOSE) AND R2A:

FOR COMPARISON OF THE FOUR MEDIA RECOMMENDED BY THE AAMI STANDARDS (1) FOR BACTERIAL MONITORING WITH TRYPTIC SOY MEDIUM OF ALMOST IDENTICAL COMPOSITION TO TSA SUPPLEMENTED WITH GLUCOSE AND WITH R2A, A PRELIMINARY 24 HOUR CULTURE WAS PREPARED AND INCUBATED AT 37°C. SAMPLES WERE STORED AT 4°C DURING THIS 24 HOUR PERIOD. BASED ON THE COUNTS FROM THE PRELIMINARY CULTURE, DILUTIONS OF THE SAMPLES WERE PREPARED IN STERILE DOUBLE DISTILLED WATER TO PRODUCE

APPROXIMATELY 100-200 CFU PER PLATE OR CASSETTE. THE DILUTE SAMPLES WERE PLATED 1.0 ML PER PLATE INTO STERILE STYRENE PETRI DISHES AND 10 ML OF THE APPROPRIATE MEDIUM ADDED AT 40-43°C. THE MILLIPORE SPC TOTAL COUNT CASSETTES WERE INOCULATED FOR 30 SECONDS WITH THE DILUTED SAMPLES ACCORDING TO THE INSTRUCTIONS OF THE MANUFACTURER. ALL SAMPLES WERE INCUBATED FOR 96 HOURS IN AN AMBIENT (23°C) HUMIDIFIED CHAMBER. WILCOXON'S RANKED SUM ANALYSIS OF RECOVERIES FROM THE DIFFERENT MEDIA IN BOTH STUDIES WAS PERFORMED ON A PC SPSS STATISTICS PACKAGE, SPSS, INC., _____.

RESULTS:

BACTERIAL CONTAMINATION OF THE THREE FLUIDS IN HEMODIALYSIS THAT POSE A RISK OF INFECTION OR ENDOTOXIC REACTIONS IN PATIENTS WERE EXAMINED ON TSBA, TSBA-HS, SMA, SMA-HS AND R2A AGAR. BICARBONATE SAMPLES, WHEN DILUTED HAD ENOUGH STERILE BCC ADDED TO MAINTAIN THE TOTAL SODIUM BICARBONATE AT A CONSTANT 1 ML PER 10 MLS OF MEDIUM (RENAL SYSTEMS FORMULATION 650 GM SODIUM BICARBONATE PER 8 L WATER). THE COUNTS PER ML FOR THE THREE FLUIDS ON THESE MEDIA ARE PRESENTED IN TABLE 1. THE RECOVERY OF BACTERIA FROM BBC IS ALMOST ALWAYS EQUAL TO OR GREATER THAN CONVENTIONAL MEDIA ON SALT SUPPLEMENTED MEDIA. A FEW SAMPLES HAD ONE LOG OR MORE HIGHER COUNTS ON SALT SUPPLEMENTED MEDIUM THAN ON THE CORRESPONDING UNSUPPLEMENTED MEDIUM. THE LEVELS OF CONTAMINATION OF BCC OBSERVED WERE NOT AS HIGH AS EXPECTED IN THE LIGHT OF RECENT REPORTS OF HIGH RISK FROM BCC CONTAMINATION (5,6,8). NONE OF THE SAMPLES SURVEYED HAD CONTAMINATION LEVELS SUFFICIENT TO PRODUCE A SIGNIFICANT BACTERIAL BURDEN IN DIALYSTATE WHEN DILUTED APPROXIMATELY 35 FOLD FIGURE 1, TABLE 1,4, EXCLUSIVE OF VERY RAPID GROWTH DURING PASSAGE THROUGH THE CONTROLLER. A NUMBER OF BCC SAMPLES (68.8%) GREW AS WELL ON R2A MEDIUM AS THEY

DID ON THE OTHER MEDIA TESTED (FIGURE 1B).

THE RECOVERY OF BACTERIA FROM WATER WAS, AS EXPECTED, ADVERSELY AFFECTED BY SALT SUPPLEMENTATION OF THE MEDIA, WITH EVERY SAMPLE SHOWING A DIMINISHED COUNT ON HIGH SALT MEDIUM RELATIVE TO THE UN-SUPPLEMENTED MEDIUM (P=). R2A AND SM MEDIA GENERALLY GAVE COMPARABLE COUNTS AND GAVE HIGHER COUNTS THAN TSBA. RANKED SUMS ANALYSIS OF THE COUNTS ON R2A AND SMA SHOWED SIGNIFICANCE/NO SIGNIFICANCE FIGURE 1 (P=).

THE MAJORITY OF DIALYSATE SAMPLES WERE ALSO NEGATIVELY AFFECTED BY SUPPLEMENTATION OF THE MEDIA WITH SALT (FIG. 1, TABLE 1, 4). SIMILAR TO WATER, R2A AND SM MEDIA GAVE COMPARABLE RECOVERY OF CONTAMINATING BACTERIA TABLE 1. R2A GAVE LOWER COUNTS THAN SM IN ONLY ONE SAMPLE OF DIALYSATE (SAMPLE 2 OF TABLE 1). BOTH GAVE HIGHER COUNTS THAN TSBA IN 15 OF 17 SAMPLES (88%), SOME ENOUGH GREATER TO BE OF CONCERN TO THE UNITS AFFECTED IF THEY USED TSBA FOR ROUTINE CULTURE. SUBSEQUENT COMPARISON OF TSA AND TSBA SHOWED COMPARABLE COUNTS ON THE TWO MEDIA FOR 9 OF 15 ANALYSES (60%, TABLE 5).

IDENTIFICATION OF BACTERIAL SPECIES IN PAIRED SAMPLINGS OF WATER, BCC AND DIALYSATE SHOWED A LACK OF CORRELATION IN THE ORGANISMS THAT CONTAMINATE DIALYSATE AND THOSE FOUND IN THE FLUIDS WHICH COMBINE TO PRODUCE THE FINAL PRODUCT IN THREE OF THE FOUR UNITS THAT WERE SURVEYED (TABLE 3). THE FOURTH UNIT APPEARS TO HAVE DERIVED ITS PRIMARY CONTAMINATION FROM THE PURIFIED WATER SUPPLY, INCLUDING THE SINGLE CONTAMINANT IN BCC. ANOTHER UNIT SURVEYED, BUT NOT SELECTED FOR BACTERIAL IDENTIFICATION, HAD A OBVIOUS CORRELATION IN THE CONTAMINATION OF DIALYSATE AND BCC, AS DETERMINED BY VERY DISTINCT COLONIAL FORMS AND COLORS (TABLE 4, CENTER 7).

AS WE REPORTED FOR WATER AND DIALYSATE IN A PREVIOUS STUDY (10), THERE WAS A LACK OF CORRELATION IN THE COUNTS OBSERVED IN

IN THE THREE FLUIDS ON ANY OF THE MEDIA OR RELATIVE TO THE HIGHEST OBSERVED RECOVERY FROM ALL OF THE MEDIA (TABLE 4). REGRESSION ANALYSIS SHOWED AN R^2 OF LESS THAN 0.82 FOR THE BEST CORRELATION (FIG. 2) WITH THE REMAINDER SHOWING AN R^2 LESS THAN 0.3. COMPARISON OF WATER AND DIALYSATE ON THE FOUR MEDIA RECOMMENDED BY THE AAMI STANDARDS WITH A MEDIUM OF ALMOST IDENTICAL NUTRIENT COMPOSITION TO TSA BUT HAVING 2.5 GM GLUCOSE PER LITER (TSBA) AND WITH R2A IS SHOWN IN TABLE 5. THE HIGH DEGREE OF VARIABILITY IN THE COUNTS OBTAINED ON THE DIFFERENT MEDIA IS THE STRIKING FEATURE OF THIS STUDY, ESPECIALLY FOR DIALYSATE SAMPLES. SM AND R2A WERE GENERALLY COMPARABLE IN RECOVERY EFFICIENCY AND WERE IN ALMOST EVERY CASE SUPERIOR TO ANY OF THE OTHER MEDIA (P=). COMPARISON OF R2A AND SMA BY RANKED SUM ANALYSIS SHOWED STATISTICAL SIGNIFICANCE/INSIGNIFICANCE (P=) DIFFERENCE IN BACTERIAL RECOVERY FOR WATER AND DIALYSATE, RESPECTIVELY. MILLIPORE SPC CASSETTES WERE POOR PERFORMERS ON BOTH WATER AND DIALYSATE COMPARED TO TSA. DIALYSATE WAS, HOWEVER, MUCH WORSE THAN WATER WITH 86.6% OF THE SAMPLES FALLING WELL BELOW TSA AND EVEN FARTHER BELOW SM OR R2A.

DISCUSSION:

RECENT REPORTS OF THE RISKS FROM BACTERIA AND ENDOTOXINS DERIVED FROM BICARBONATE CONCENTRATES OF COMMERCIAL OR LABORATORY ORIGIN (5,6,8) AND THE ADVENT OF HIGH PERMEABILITY DIALYSIS MEMBRANES HEIGHTENS CONCERN FOR THE QUALITY OF DIALYSIS FLUID. WE REPORTED PREVIOUSLY THAT THE TRADITIONAL MEDIA EMPLOYED FOR TESTING DIALYSIS FLUIDS MAY BE TOTALLY INADEQUATE IN MONITORING PURIFIED WATER, USED FOR PREPARATION OF DIALYSATE (3). THE ASSUMPTION THAT WATER WOULD BE THE LARGEST CONTRIBUTOR TO THE CONTAMINATION OF DIALYSATE MAY BE ERRONEOUS, IN THE LIGHT OF THE REPORTS CITED ABOVE. BLAND ET.

AL. (5) SUGGESTED, FROM THEIR STUDY THAT SUPPLEMENTATION OF MEDIA WITH NACL AND/OR BICARBONATE WERE NECESSARY FOR MAXIMUM RECOVERY OF BACTERIA FROM DIALYSATE. IF THIS OBSERVATION IS CONFIRMED, IT SUGGESTS THAT BBC MAY BE A MAJOR CONTRIBUTOR TO DIALYSATE CONTAMINATION AND THAT THE ENTIRE PROCESS OF MONITORING DIALYSIS CENTER CONTAMINATION MAY NEED RE-THINKING. THESE OBSERVATIONS SUGGESTED THAT TRACKING OF THE CONTAMINATION PROCESS SHOULD BE ACCOMPLISHED AND THAT A SYSTEMATIC COMPARISON OF MEDIA WAS IN ORDER. FIRSTS, WE COMPARED PAIRED SAMPLINGS OF WATER, BBC AND DIALYSATE FROM INDIVIDUAL PRO-PROTIONERS, TO ASSESS THE RELATIVE CONTRIBUTIONS OF WATER AND BCC TO THE CONTAMINATION OF DIALYSATE. DATA FROM RANDOM SAMPLINGS OF THE THREE FLUIDS WERE ALSO INCLUDED IN THE ANALYSIS OF THE INFLUENCE OF THE DIFFERENT MEDIA. THE ACID CONCENTRATE USED TO FORMULATE BICARBONATE DIALYSATE IS OF TO LOW PH AND TOO HIGH OSMOLARITY TO SUPPORT SIGNIFICANT BACTERIAL GROWTH (5) AND, THEREFORE, WAS NOT INCLUDED IN THE STUDY. WE TESTED THE THREE FLUIDS ON FIVE DIFFERENT MEDIA, CHOSEN TO COMPARE THE RELATIVE RECOVERY OF CONTAMINATING ORGANISMS FROM THE FLUIDS AND FOR COMPARISON OF THE RELATIVE LEVELS OF CONTAMINATION IN THE FLUIDS. WE CONCUR WITH BLAND AND CO-WORKERS AND WITH EBBEN AND CO-WORKERS (5,6) THAT BCC SHOULD BE CULTURED ON SALT SUPPLEMENTED MEDIUM TO ASSURE OPTIMUM RECOVERY. HOWEVER, WE DID NOT FIND SALT SUPPLEMENTED TSBA OR SM ROUTINELY BETTER THAN THE SAME MEDIA WITHOUT ADDED SALT, ONLY 2 OF 14 SAMPLES (14%) HAD MORE THAN ONE LOG HIGHER COUNTS. SINCE WE USED POUR PLATE TECHNIQUE EXCLUSIVELY, WE WOULD HAVE 344.8 MM NACL IN OUR SALT SUPPLEMENTED MEDIA AND APPROXIMATELY 127.4 MM SODIUM BICARBONATE IN ALL OUR PLATES BBC ANALYSES (BASED ON RENAL SYSTEMS FORMULATION 650 GM BICARBONATE IN 8 L WATER AND 1 ML BCC IN 10 MLS MEDIUM). WHILE WE DID NOT COMPARE

MORE DILUTE SAMPLES OF BCC, THERE WAS A MODEST INCREASE IN BACTERIAL COUNTS IN A FEW SAMPLES WHEN TSBA OR SMA WERE SUPPLEMENTED WITH 2% NACL. IT IS POSSIBLE THAT THE BCC CONCENTRATION IN OUR POUR PLATES WAS ADEQUATE TO STIMULATE ALMOST OPTIMUM GROWTH AND IT IS POSSIBLE THAT SALT SUPPLEMENTED TSA WOULD HAVE PERFORMED BETTER THAN TSBA. IN CONTRAST TO EBBEN ET. AL. (6), WE FOUND THAT SMA WAS AN ADEQUATE MEDIUM FOR RECOVERY OF BCC CONTAMINANTS AS COMPARED TO TSBA. ADDITION OF 2% NACL MADE BOTH SUPERIOR, FOR SOME SAMPLES, TO THE NON-SUPPLEMENTED MEDIA. WE FOUND A SURPRISING NUMBER OF BCC SAMPLES THAT GREW WELL ON R2A MEDIUM. THIS SUGGESTS THAT THE ORGANISM THAT CONTAMINATED OUR SAMPLES ARE PROBABLY MORE ACCURATELY DESCRIBED BY EBBEN AND CO-WORKERS (6), IE. THAT THEY ARE HALOTOLERANT ORGANISMS. THIS IS SUPPORTED BY THE FACT THAT ALL OF THE ORGANISMS PICKED FOR IDENTIFICATION WERE GROWN ON TSBA OR R2A FOR ISOLATION. THE FATE AND INFLUENCE OF THESE ORGANISMS ON DIALYSATE ARE A PART OF OTHER STUDIES IN PROGRESS.

SURPRISING, WERE THE NUMBER OF BCC SAMPLES THAT HAD SUCH LOW CONTAMINATION LEVELS THAT THEY COULD NOT BE INCLUDED IN THE COMPARISON (ALL MEDIA GAVE COUNTS BELOW THE THRESHOLD COUNT OF 20 CFU/ML SEE TABLE 4). IT DOES NOT APPEAR THAT THE UNDER-ESTIMATION OF CONTAMINATION OF CENTER PREPARED BCC WOULD BE A SIGNIFICANT PROBLEM IF TRADITIONAL MEDIA ARE EMPLOYED. THE COUNTS OBSERVED IN OUR BCC SAMPLES WERE GENERALLY SO LOW THAT THE 35 FOLD DILUTION REQUIRED BY MOST DIALYSIS PROPORTIONERS, WOULD MAKE THE CONTRIBUTION TO DIALYSATE A NEGLIGABLE PROPORTION OF THE AAMI CONTAMINATION LIMIT. WE CONCLUDE THAT CENTERS THAT PREPARE THEIR BBC IN HOUSE AND MAINTAIN GOOD SANITIZATION PROCEDURES SHOULD NOT INCUR SIGNIFICANT CONTAMINATION FROM THIS SOURCE. THERE IS THE POTENTIAL, HOWEVER, FOR RAPID GROWTH

OF THESE SALT TOLERANT ORGANISMS AND FOR THEIR TRANSMISSION TO DIALYSATE (TABLE 4, CTR 7). THE COLONIES OF BOTH BCC AND DIALYSATE IN THESE SAMPLES WERE SO DISTINCT THAT IT WAS OBVIOUS THAT THE PRIMARY CONTAMINATION OF DIALYSATE ORIGINATED IN THE BBC AND PRODUCED COUNTS ON SM OR R2A IN EXCESS OF AAMI TOLERANCE.

OUR ANALYSIS OF SALT SUPPLEMENTATION OF DIALYSATE CULTURE MEDIUM ARE IN CONTRAST TO THOSE REPORTED BY BLAND AND CO-WORKERS (5). WE OBSERVED A NEGATIVE EFFECT ON DIALYSATE WHEN CULTURED ON SALT SUPPLEMENTED MEDIA 4 OF 17 ON TSBA (76%) AND 6 OF 17 ON SMA-HS (65%) (TABLE 1). SINCE THE SALT CONTRIBUTION OF THE DIALYSATE TO THESE CULTURES WAS NEVER MORE THAN 30 M OSMOLES (1ML SAMPLE IN 10 ML MEDIUM), THE SALT SUPPLEMENTED MEDIA WOULD HAVE MAXIMAL EFFECT. THIS OBSERVATION IS QUITE REASONABLE SINCE THE CONTAMINATING ORGANISMS IN BCC WOULD BE DILUTED 35 FOLD IN PREPARATION OF THE DIALYSATE AND MAY BE OSMOTICALLY SHOCKED OR INHIBITED BY OTHER CONTAMINATING SPECIES FROM THE WATER OR OTHER SOURCES. CONTAMINATING ORGANISMS FROM OTHER SOURCES (WATER, PERSONNEL, AIR BORN) WOULD NOT BE HALODURIC AND POSSIBLY NOT HALOTOLERANT. THE TOTAL CHANGE IN OSMOTIC PRESSURE ON BCC CONTAMINANTS DUE TO THE DILUTION INTO THE SALT SUPPLEMENTED PLATES WAS APPROXIMATLEY 1:2 (970 MM FOR BCC TO 470 MM IN THE PLATE EXCLUSIVE OF THE MEDIUM COMPONENTS).

WE ALSO OBSERVED THAT EVERY COLONY PICKED FROM HIGH SALT CULTURES OF BCC COULD BE GROWN ON TSA AND/OR R2A FOR PURPOSES OF ISOLATION AND IDENTIFICATION. THE BACTERIA APPARENTLY ADAPT READILY TO A LOWER SALT ENVIRONMENT AND CAN GROW WELL ON THE UNSUPPLEMENTED MEDIA.

THE SUPERIOR RECOVERY OF CONTAMINANTS OF WATER AND DIALYSATE ON STANDARD METHODS AGAR AND R2A IN THIS RANDOM SAMPLING OF NINE DIFFERENT DIALYSIS CLINICS IN KENTUCKY AND INDIANA SUGGESTS THAT

THESE MEDIA SHOULD BE ESTABLISHED AS THE STANDARD MEDIA FOR MONITORING THESE FLUIDS. THE POOR RECOVERIES OBTAINED WITH DIPSTICK CASSETTES INDICATES THAT THE CONVENIENCE OF THESE DEVICES SHOULD BE BALANCED AGAINST THE POTENTIAL RISKS THAT MAY BE INCURRED TO THE PATIENT FROM UNDERESTIMATION OF THE BACTERIAL BURDEN. IT APPEARS THAT THE MONITORING OF THE BACTERIAL BURDEN IN CENTER PREPARED BCC MAY BE DONE ON EITHER TSA OR SM PLATES, WITH OR WITHOUT SALT SUPPLEMENTATION WITHOUT SIGNIFICANT RISK TO PATIENTS. IT SI CRITICALLY IMPORTANT THAT GOOD SANITIZATION PROCEDURES BE FOLLOWED IN MAINTAINING GOOD CONCENTRATE QUALITY.

THE LACK OF CORRELATION IN BACTERIAL IDENTITY ACROSS THE FLUIDS THAT BRING A RISK TO THE DIALYSIS CENTER AND THE LACK OF CORRELATION OBSERVED IN OUR PREVIOUS STUDY (10) AND IN THE CURRENT STUDY, SUGGEST THAT CONTAMINATION OF THESE FLUIDS MAY NOT ARISE IN A SEQUENTIAL MANNER. IT SUGGESTS THAT THE DIALYSIS PROPORTIONER COULD BE CONTAMINATED FROM OTHER SOURCES, SUCH AS, INADEQUATE ASEPTIC TECHNIQUE IN OPERATION OF THE MACHINE. THIS IS FURTHER SUPPORTED BY REPEATED OBSERVATION OF VERY HIGH CONTAMINATION LEVELS IN ONE OF THE CENTRAL DIALYSATE PROPORTIONERS WHERE THE WATER WAS VERY CLEAN. BBC SAMPLES WERE NEVER COLLECTED FROM THIS CENTER, SINCE IT WAS INCLUDED ONLY IN THE SECOND PHASE OF THE STUDY, THEREFORE, A COMPARISON OF ALL OF THE FLUIDS CANNOT BE MADE. ADDITIONAL STUDIES NEED TO BE PURSUED TO DETERMINE THE SOURCES, RISKS AND STRATEGIES NEEDED TO DECREASE THE BACTERIAL BURDEN OF DIALYSATE EMPLOYED IN THIS LIFE SAVING THERAPY.

TABLE 1

TSBA-HS/TSBA	SMA/TSBA	SMA-HS/TSBA	R2A/TSBA
WATER:			
0	36.50	0.15	44.21
0	19.46	0.58	11.69
0.12	1.21	0.26	0.96
0.01	1.40	0.03	2.21
0	3.75	0.05	3.86
0.48	2.54	1.01	2.12
0	1.39	0.57	0.74
0	2.00	0.02	8.40
0.06	2.88	0.22	6.63
0.17	5.39	0.06	14.11
0.04	2.47	0.18	2.94
0	2.63	0.01	2.93
DIALYSATE			
0	2.06	0.02	2.03
0.01	1.17	0.14	0.68
0.71	1.19	0.52	1.26
0.01	1.14	0.03	1.97
0.04	1.17	0.22	1.22
0.68	0.49	0.80	0.62
0.82	1.06	1.10	1.32
0.56	1.48	1.53	1.95
1.00	0.21	0.92	1.08
0.06	1.82	0.17	4.39
1.00	0.21	0.92	1.08
0.06	1.82	0.17	4.39
0.02	1.36	0.19	1.48
0.18	32.36	0.21	49.11
0.02*	2.32	0.30	2.62
0*	19.69	0	37.56
1.00*	14.00	1.00	27.00
SCC			
0.41	0.94	0.93	1.01
1.17	2.08	1.00	1.58
13.46	3.19	18.98	9.56
1.07	0.07	1.04	0.10
16.94	2.06	14.59	2.82
1.03	0.65	0.97	0.72
1.10	1.05	1.04	1.11
0.69	0.77	0.93	0.88
0.81	0.81	1.00	0.87
1.37	0.88	2.76	2.12
1.01	0.91	0.87	0.75
0.77	0.64	0.85	0.92
1.29	2.14	0.71	3.29
0.78	0.44	0.78	1.11
p=	p=	p=	p=

*Acetate dialysates.

TABLE 1

TSBA-HS/TSBA	SMA/TSBA	SMA-HS/TSBA	R2A/TSBA
WATER:			
0	36.50	0.15	44.21
0	19.46	0.58	11.69
0.12	1.21	0.26	0.96
0.01	1.40	0.03	2.21
0	3.75	0.05	3.86
0.48	2.54	1.01	2.12
0	1.39	0.57	0.74
0	2.00	0.02	8.40
0.06	2.88	0.22	6.63
0.17	5.39	0.06	14.11
0.04	2.47	0.18	2.94
0	2.63	0.01	2.93
DIALYSATE			
0	2.06	0.02	2.03
0.01	1.17	0.14	0.68
0.71	1.19	0.52	1.26
0.01	1.14	0.03	1.97
0.04	1.17	0.22	1.22
0.68	0.49	0.80	0.62
0.32	1.06	1.10	1.32
0.56	1.48	1.53	1.95
1.00	0.21	0.92	1.08
0.06	1.82	0.17	4.39
1.00	0.21	0.92	1.08
0.06	1.82	0.17	4.39
0.02	1.36	0.09	1.48
0.18	32.36	0.21	49.11
0.02*	2.32	0.03	2.62
0*	19.69	0	37.56
1.00*	14.00	1.00	27.00
TCC			
0.41	0.94	0.76	1.01
1.17	2.06	1.00	1.58
11.46	2.19	18.78	9.58
1.07	0.07	1.04	0.10
14.94	2.06	14.59	2.82
.03	0.65	0.97	0.72
1.10	1.05	1.04	1.11
0.69	0.77	0.93	0.88
1.01	0.81	1.00	0.87
1.37	0.59	2.76	2.12
1.01	0.91	0.87	0.75
.77	0.64	0.85	0.92
1.24	2.14	0.71	3.29
0.78	0.44	0.78	1.11
p=	p=	p=	p=

*Calculated with placebo.

TABLE 3

BACTERIA IDENTIFIED IN PAIRED SAMPLES OF WATER, BICARBONATE AND DIALYSATE

WATER	CONCENTRATE	DIALYSATE
Center 1		
Pseudomonas paucimobilis		**Pseudomonas paucimobilis**
Pseudomonas stutzeri	**Pseudomonas stutzeri**	
Pseudomonas picketti		Pseudomonas luteola
Alcaligenes denitrificans	Achromobacter sp.	Pseudomonas vesicularis
Aeromonas salmonicida		Agrobacter radiobacter
		Aeromonas hydrophilia
		Moraxella sp.
Center 2		
Moraxella sp.		**Moraxella sp.**
Pseudomonas paucimobilis		**Pseudomonas paucimobilis**
Achromobacter gr. V	Pseudomonas oryzihabitans	CDC GR. IV E
	Pasturella haemolytica*	
	*tentative ID	
Center 3		
Pseudomonas cepacia		**Pseudomonas cepacia**
Pseudomonas paucimobilis		**Pseudomonas paucimobilis**
Corynebacterium sp.	**Corynebacterium sp.**	
	Pseudomonas maltophilia	Pseudomonas maltophilia
	Acinetobacter calco lwoffi	
	Moraxella sp.	CDC GR IV C-2
	Pseudomonas stutzeri	
	G- bacillus	
Center 4		
Pseudomonas luteola	**Pseudomonas luteola**	**Pseudomonas luteola**
Pseudomonas cepacia		**Pseudomonas cepacia**
Pseudomonas paucimobilis		**Pseudomonas paucimobilis**
Pseudomonas dimunita		**Pseudomonas dimunita**
Pseudomonas fluorescens		Pseudomonas vesicularis

TABLE 4

	TSBA	TSBA-HS	SMA	SMA-HS	R2A
Center 1A	CFU/ml				
Water	13	0	253	8	152
BCC	1137	468	1066	1054	1147
Dial.	132	1	154	19	90
Center 2A					
Water	678	0	24750	104	29975
BCC	0	0	0	3	1
Dial.	1062	0	2185	22	2160
Center 2B					
Water	2142	0	4289	49	18000
BCC	4	5	8	3	12
Dial.	30650	30570	6360	28340	33110
Center 3					
Water	57	7	69	15	55
BCC	12	14	25	12	19
Dial.	31	22	37	16	39
Center 4					
Water	41	20	104	42	87
BCC	880	889	803	768	659
Dial.	244	199	259	268	321
Center 5A					
Water	390	1	1024	2	1141
BCC	0	0	0	0	0
Dial.	28	5	906	6	1375
Center 5B					
Water	36	6	194	1	508
BCC	0	0	0	0	0
Dial.	5	5	70	3	85
Center 6					
Water	554	24	1366	100	1626
BCC	0	0	0	0	0
Dial.	550	10	750	105	815
Center 7					
Water	4	1	12	0	42
BCC	2032	1066	2273	1742	2338
Dial.	4880	1540	8410	5770	7400
Center 8					
Water	353	23	1015	77	2340
BCC	1	0	10	0	7
Dial.	1265	70	2305	213	5550

TABLE 5

DIALYSATE B1/TSA	SM/TSA	TSBA/TS	R2A/TSA	Mill./TSA
0.780	0.639	0.917	0.936	0.135
0.955	0.840	0.861	0.885	0.357
1.066	0.808	0.960	0.953	0.060
0.918	1.166	1.024	1.257	0.760
0.877	0.855	0.879	0.976	0.352
0.850	0.594	1.094	0.936	0.033
1.067	0.133	0.933	0.333	0.200
1.625	2.500	0.375	6.625	0.500
1.375	1.109	0.375	1.563	1.469
0.853	1.078	1.144	1.184	0.775
0.864	0.615	0.464	0.996	0.328
1.054	24.486	0.757	37.162	8.595
0.368	2.000	0.263	3.632	0.000
1.229	1.042	0.521	1.875	0.813
0.689	7.000	0.356	13.356	0.711
WATER				
0.911	5.036	1.393	7.286	1.607
0.955	0.840	0.861	0.885	0.357
0.440	1.406	1.047	1.809	0.176
0.590	1.452	0.303	1.618	0.563
0.873	1.229	0.578	1.367	0.680
0.927	1.209	0.887	0.957	1.080
0.348	1.130	0.783	3.652	0.435
0.833	1.269	0.500	2.241	0.593
0.837	2.496	0.574	3.255	1.234
0.815	2.444	0.556	2.444	0.667
0.964	1.099	0.418	1.224	1.095

FIGURE LEGENDS

Figure 1.

Colony forming units (CFU/ml) are plotted for TSBA , TSBA-HS , SMA , SMA-HS and R2A . The samples through center 8 are paired samples of which four were selected for identification of all species present in the three fluids. Centers not represented in BCC (panel B) had zero counts for all media.

Figure 2.

CFU/ml for water and BCC are plotted versus CFU/ml for dialysate for cultures grown on TSBA. Linear regression analysis of the counts showed no correlation of counts for either water or BCC with dialysate contamination levels (r^2=0.82 and 0.007 for water and BCC on TSBA respectively). Values for other media and for maximum counts from all media gave correlations no greater than 0.3.

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